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Therapeutic Applications for Ligands of Retinoid Receptors

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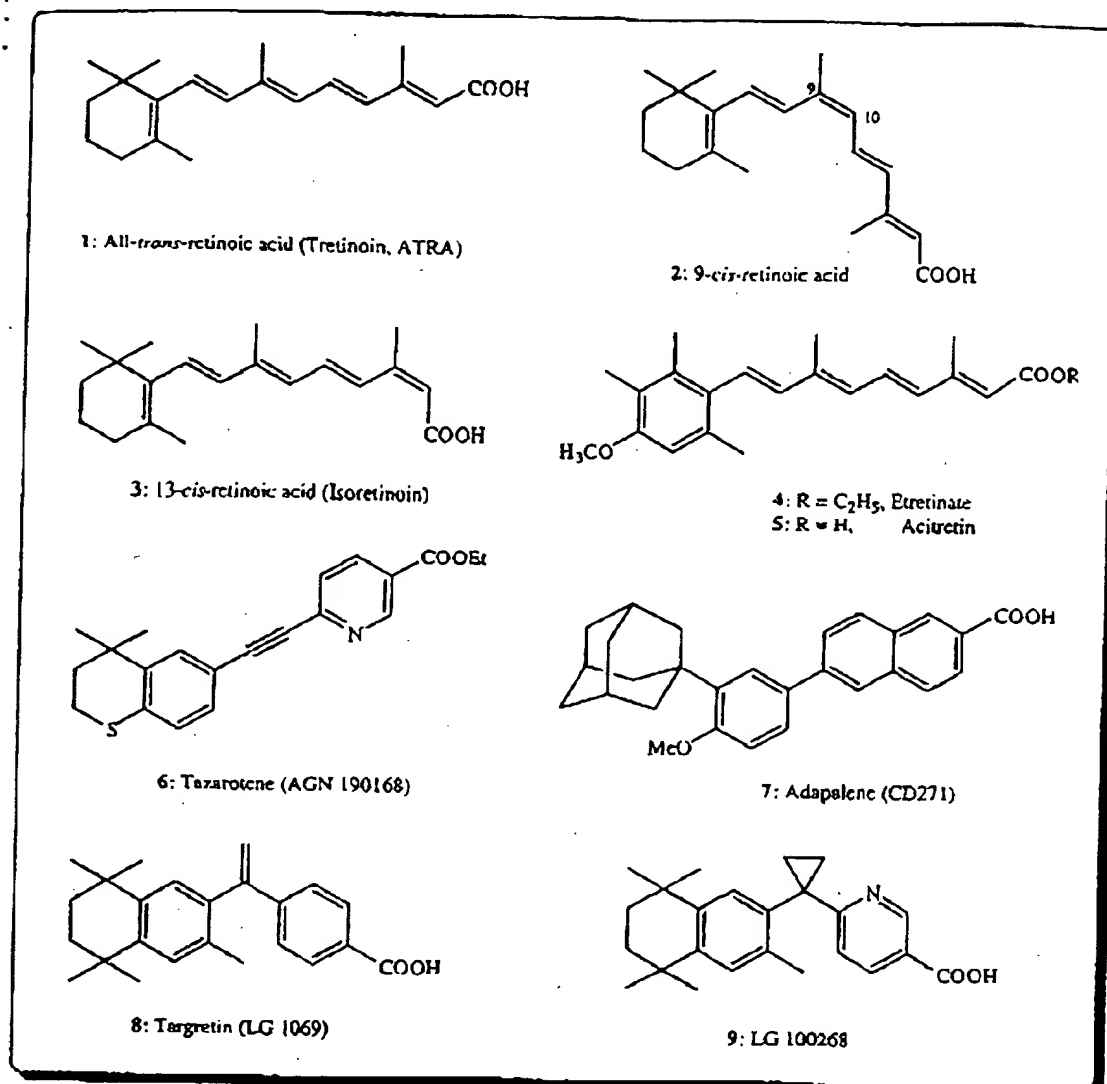
Abstract: Synthetic retinoids, ligands for the RAR and RXR members of the steroid/thyroid superfamily of nuclear hormone receptors, are used for the treatment of psoriasis, acne, photoaging and cancer. Retinoid mechanisms of action for these conditions largely involve effects on epithelial differentiation and modulation of inflammation with, some impact on the immune system. Retinoid medicinal chemistry in recent years has identified ligands highly specific for one of the three RAR subtypes (RAR-α) and for the RXR family of receptors, as well as antagonists for the RARs, RARα and the RXRs. Structure-activity relationships among the novel retinoid classes are reviewed along with potential therapeutic activities and side effects. RAR-α specific retinoids inhibit cancer cell growth but lack other retinoid toxicities, including skin irritation now ascribed to RAR-γ. RXR-specific retinoids lower blood glucose in animal models of type 2 diabetes albeit with a potential for mild hypothyroidism. Function-selective retinoids, especially a class of RAR antagonists called inverse agonists, have unexpected gene regulatory activity. Given the diverse properties and tissue distributions of the retinoid receptors, synthesis of additional classes of receptor-specific and function-selective ligands has the potential to produce novel therapeutic applications.

1. INTRODUCTION

Retinoids are naturally occurring and synthetic derivatives of retinol (Vitamin A) which bind one or more of the six RAR and RXR nuclear receptors for all trans retinoic acid 1 (ATRA) and 9-cis RA 2. Retinoids have a remarkable range of activities and regulate, among other things, embryonic development, bone formation, cell differentiation and proliferation, glucose and lipid metabolism, carcinogenesis, and immune function. They have an equally impressive range of clinical applications. Current retinoid therapies include: (1) treatment success rates of ≥60% in the severest form of inflammatory acne, nodulocystic acne, following a single 3-4 month course of oral 13-cis RA (isotretinoin) 3 [1]; (2) differentiation of the rare neoplasm, acute promyelocytic leukemia (APL), by ATRA, resulting in ~85% complete remission with follow-up cytotoxic chemotherapy [2]; (3) significant improvement in psoriasis with the oral retinoids etretinate 4 and acitretin 5 and a topical retinoid (tazarotene 6), providing significant improvement and long-term remission in stable plaque psoriasis [3]; (4) prevention of second head and neck cancers by 13-cis RA after treatment of an initial carcinoma [4]; (5) topical therapy of acne vulgaris using ATRA, tazarotene, or adapalene 7 [5]; and (6) reversal of histological and morphological changes to skin due to UV-mediated long-term photodamage with ATRA [6]. RXR-selective retinoids such as LG 1069 8 and LG 100268 9 are also predicted to be therapeutically useful in treatment of diabetes based on their blood glucose lowering effects in animal models of the disease [7,8]. Limiting retinoid dosage are receptor-mediated adverse effects that include irritation and inflammation to skin and mucous membranes [9], elevation of serum triglycerides [10], dysregulation of bone formation and resorption [11], headache, hypothyroidism [12], and fetal malformations [13]. One goal of retinoid medicinal chemistry is the design of ligands selective for individual retinoid receptors which will have a narrower range of side effects while maintaining specific therapeutic retinoid activities. A second goal is to design "function-selective" retinoids, compounds that are antagonists or partial agonists and have novel gene regulatory properties and hence new therapeutic applications [14-16].

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EXHIBIT 1

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1: All-trans-retinoic acid (Tretinoin, ATRA)

2: 9-cis-retinoic acid

3: 13-cis-retinoic acid (Isoretinoin)

4: R = C₂H₅, Etretinate
5: R = H,   Acitretin

6: Tazarotene (AGN 190168)

7: Adapalene (CD271)

8: Targretin (LG 1069)

9: LG 100268

2. THE RETINOID RECEPTORS

The pharmacological targets of all-trans and 9-cis RA, the retinoic acid receptors (RAR-α, β, and γ) and the retinoid X receptors (RXR-α, β, and γ), are DNA binding proteins that regulate gene transcription in response to ligand binding. Their structures are similar to other members of the nuclear receptor superfamily that includes the steroid and thyroid hormone receptors [17]. All of the receptors have a modular structure with distinct domains for DNA-binding, ligand binding, and activation of gene transcription. Despite these similarities, the RARs and RXRs differ in several very significant ways. All-trans RA and its isomer 9-cis RA bind the RARs with high affinity ($K_D \sim 1$ nM), whereas the RXRs bind only 9-cis RA

[18,19]. The two receptor types also differ markedly in their mechanisms of DNA binding and regulation of gene transcription. The RARs do not bind DNA alone, but must form a 1:1 complex with an RXR. This heterodimer recognizes a direct repeat of a 6 base-pair DNA sequence motif with a 2 or 5 base pair spacer (DR2 and DR5) that is found in the promoter of many retinoid-inducible genes [17,20]. The RXR partner of the RAR/RXR complex appears to be inactive in vivo and in normal cell cultures since RXR-specific ligands cannot replicate the activities of RAR-specific ligands [10,21-24]. In certain cultured cells, however, the full response to all-trans RA can only be obtained if ligands specific for the RAR and RXR receptors are added simultaneously, indicating that RXR can be activated if the RAR

partner is occupied [20,25]. RXR-specific ligands have significant in vivo activity unrelated to the RARs, including lowering of serum glucose and triglycerides in diabetic animals and induction of som enzymes of lipid oxidation [8,26,27]. The metabolic effects of RXR ligands probably occur through one or more non-RAR/RXR heterodimers. RXRs are required heterodimeric partners for a growing list of nuclear receptors, including thyroid receptor (TR), PPAR-α and PPAR-γ which regulate lipid metabolism, and the oxysterol receptors LXRα and FXR which regulate cholesterol metabolism [28-31]. Two examples of heterodimeric partners for RXR which transduce RXR ligand action efficiently, in contrast to the RARs, are PPAR-γ, which is a receptor for the thiazolidinedione (TZD) class of drugs that lowers blood glucose in diabetics [32], and PPAR-α, which induces fatty acid β-oxidation [28,33]. Although RXRs can form homodimers in vitro, they are generally associated with other heterodimeric partners in vivo [34]. The diversity of RXR heterodimers that respond to RXR ligands suggests that there may be a wide range of therapeutic modalities for RXR ligands [35].

a. Tissue Distribution and Function

The critical assumption behind a strategy of receptor-selective drug design is that each of the RARs and RXRs have distinct physiological functions. This is well substantiated by differences in tissue distribution and function of the receptors [17,36-39]. For example, RAR-α is present in the majority of tissues while the distribution of RAR-β and γ is more selective [40]. In epidermis, RAR-γ is the most highly expressed whereas RAR-α is present at much lower levels and RAR-β is essentially absent [17,41], and these findings correlate well with the observations that major retinoid-mediated effects in epidermis (irritation and hyperplasia) are mediated by ligand binding to RAR-γ [42,43]. RAR-γ is also expressed in developing bone while RAR-β is strongest in surrounding mesenchyme, suggesting dual roles for retinoids in skeletal development [40]. RAR-β is also highly expressed in several tissues such as lung where it may function as a negative regulator of neoplasia [44,45]. Equally significant disparities are observed in the distribution of the RXRs. RXR-β is the most widely distributed, whereas RXR-α is expressed predominantly in liver, intestine, and is the major RXR in skin [17]. RXR-γ is more selectively expressed in muscle and certain parts of the brain and pituitary [38,46]. Homozygous knockouts for the individual receptors are generally

viable, except for RXR-α null mutants which die in utero due to defects in the heart [47], suggesting a large degree of overlapping function between receptors in the knockout animals. Some specific defects in the null mice are of interest. RAR-γ null mice have extensive skeletal defects and squamous metaplasia of several epithelia, corresponding to effects of Vitamin A deficiency. A separate symptom of Vitamin A deprivation, inhibition of spermatogenesis [47], is observed in RAR-α knockout mice. RAR-β/RXR-α double knockouts have defects in spatial learning [38] and RAR-β null mice crossed with RXRβ or RXRα knockouts have defects in locomotion that may be related to the reduced expression of dopamine receptors [48], suggesting that some selective retinoids may potentially alter neuronal signaling capacity in pathological conditions. Gene transfection studies also suggest that individual receptors can have distinct activities even within the same cell. For example, the individual subtypes of the RARs or RXRs show quantitatively different patterns of induction of gene expression when compared at multiple promoters [39]. These differences are ascribed to the N-terminal activation function, 1 (AF-1) domains of the receptors, which, in contrast to the DNA- and ligand-binding domains, are quite distinct among the members of each receptor family [49].

b. Receptor Co-Regulatory Factors

Comparative X-ray crystallographic studies of the ligand binding domains of (unliganded) RXR-α (apo-RXR) and of holo-RAR-γ (with ATRA bound) demonstrate that ligand binding produces a profound conformational change [50]. The conformational change reorients helix 12 of the ligand-binding domain so that its activation function-2 (AF-2) region, which is required for ligand-dependent induction of gene expression, can bind one of several transcriptional co-activators [51]. Transcriptional co-activators of the homologous p160 family, SRC-1/NCoA-1, TIF2/NCoA-2, and pCIP/ACTR, bind the AF-2 loop of several members of the nuclear receptor family through motifs containing the consensus sequence LXXLL [52]. These co-activators also express histone acetyltransferase (HAT) activity, which relaxes chromatin structure by acetylation at lysine residues as a prerequisite for binding of cellular transcriptional machinery. In addition to the presence of HAT activity, an earlier displacement of linker H1 histones must be achieved in order to effect an unfolding of the compact chromatin structure. We have recently shown that RARs

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interact in a ligand-dependent manner with high mobility group protein-I (HMG-I) which is known to displace H1 histone from chromatin [53]. These findings suggest a general stepwise mechanism by which transcription factors such as RARs can cause an unfolding of compact chromatin by recruitment of HMGs and then HAT activity. Two transcriptional co-repressors, N-CoR and SMRT [54,55], are released upon ligand binding to RARs and RXRs. These have highest affinity for the receptors in their unliganded state and bind along α-helix 1 of the RAR ligand-binding domains. In contrast to the co-activators, the co-repressors recruit the histone de-acetylase activity, mSin3A [56], a homologue of a yeast global repressor of transcription and a functional element of many other transcriptional repression complexes. These data suggest that chromatin remodeling by selective histone modification is a key step in RAR and RXR modulation of gene expression. For the purposes of drug design, it is intriguing that certain ligands which bind equally well to the isolated LBDs (ATRA and 9-cis RA in the case of RAR-α) are reported to discriminate among interacting co-factors, such as SRC-1 [57], suggesting that, depending on the tissue specificity of co-factor expression, functional selectivity may be obtained among agonists.

3. SELECTIVE AGONISTS AND ANTAGONISTS OF THE RARS AND RXRS

Several conformationally restricted analogues of ATRA have been made with significant success in achieving subtype as well as function selectivity at RAR. RXR specific compounds have also been synthesized, and some were characterized essentially simultaneously with the identification of 9-cis-retinoic acid 2 as the putative hormone for the RXRs [58-60]. The following sections outline some of the structural requirements for individual RAR subtype selectivity and the structural differences between RAR and RXR selective ligands from systematic structure-activity relationship (SAR) studies with the individual RAR and RXR receptors.

a. RAR Agonists

The structure of ATRA can be divided into a left-hand hydrophobic region A comprising a cyclohexenyl ring, a polyolefinic chain B and a polar head group C (Fig. 1). The polyolefinic chain can be viewed as a tether region between the
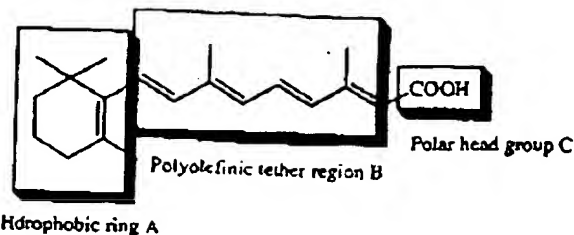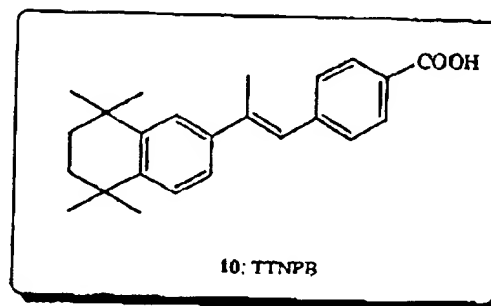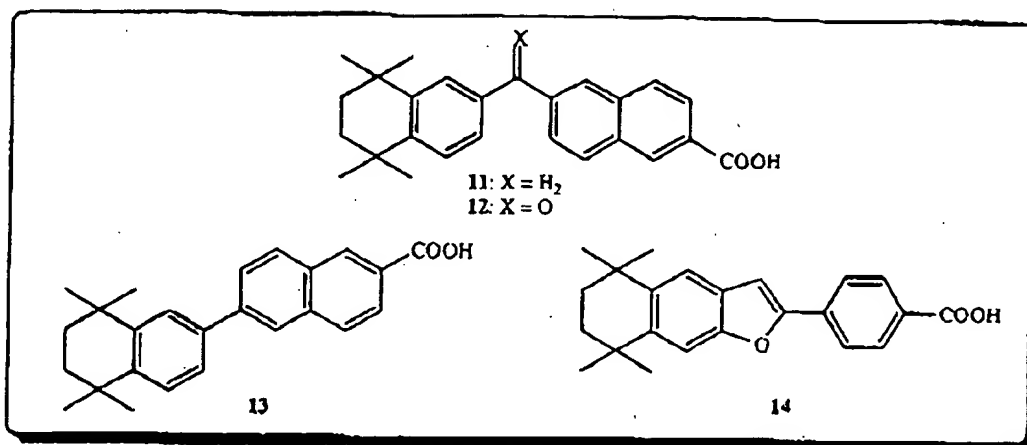

Fig. (1). All-trans-retinoic acid.

cyclohexenyl ring and the carboxylic acid. An early example of a conformationally restricted analogue of ATRA with potent RAR panagonist activity is the stilbene-based retinoid TTNPB 10 [61]. The tether region in this molecule is a doubly bonded two-carbon unit. The tetramethyl tetralin unit constitutes the hydrophobic region and the 4-substituted benzoic acid moiety contains the polar head group. The trans-double bond of the tether region corresponds to the 9-trans-double bond of retinoic acid, hence the observed similarity to ATRA in receptor binding profile. The tether region is very important in determining the subtype selectivity of compounds as well as for RAR vs RXR specificity as will be seen in the following sections.
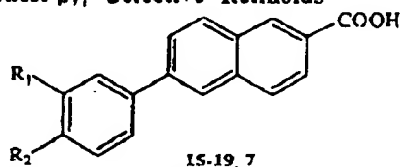

10: TTNPB

(i) RAR-β,γ Selective Retinoids

The 6-substituted 2-naphthoic acids 11 and 12 were shown to activate gene transcription at RAR-β and γ but not at RAR-α [62]. Other examples of such compounds are the bisnaphthalene derivative 13 [63] and the anthracene-like analog 14 [64]. Modification of the left-hand lipophilic portion of the bisnaphthalene retinoid 13 led to the discovery of compounds 15-16, 7 and 17-19 (Table 1) that were either RAR-β or RAR-γ selective [65]. The 4-hydroxy substituted derivatives 17-19 are RAR-γ selective with increasing binding affinity at RAR-γ as the hydrophobic group at the 3-position increases in size from a t-butyl group to a 1-adamantyl group. On the other hand, the 4-methoxy substituted derivatives 15-16, 7 are moderately

11: X = H₂
12: X = O

13

14

RAR-β selective. The 4-methoxy substituted retinoid 7 (CD 271, adapalene), in currently marketed as a topical agent for the treatment of acne.

A series of acetylenic retinoids 20-24 and the esters 25 and 26 kept the cyclohexenyl ring of ATRA intact while varying the tether to a rigid acetylene and the right hand portion to various
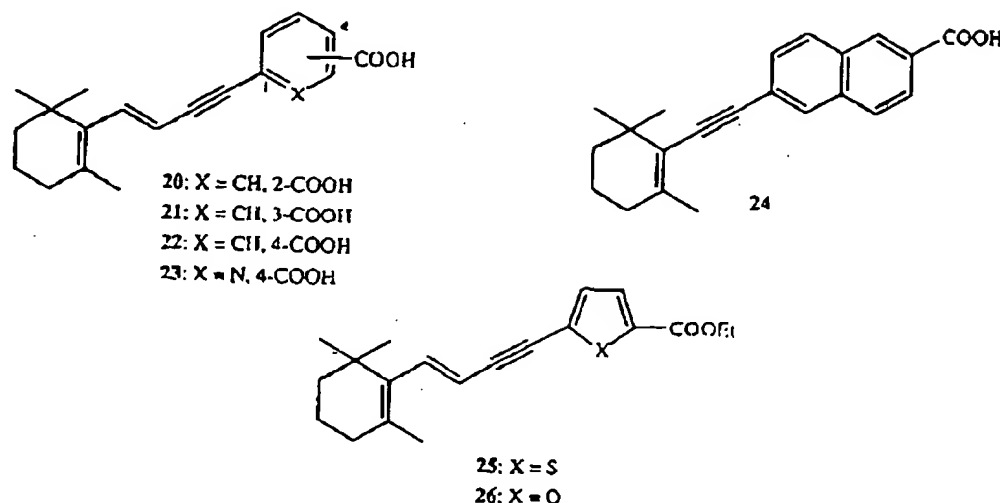
Table 1.   Binding Affinities of RAR-β,γ Selective Retinoids

	Substitution		K_i (nM)			F9 Differentiation
	R_1	R_2	RAR-α	RAR-β	RAR-γ	AC_{50} (nM)
ATRA			15	13	18	200
15		OMe	6500	36	426	200
16		OMe	1120	26	160	150
7 (CD 271)		OMe	1100	34	130	37
17		OH	NA[1]	3550	200	NA
18		OH	NA	660	121	175
19		OH	6500	2480	77	33

Results are the mean of three experiments. NA = Not Active

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Table 2.   Transcriptional Activation Assay Data of Acetylenic Retinoids

20: X = CH, 2-COOH
21: X = CH, 3-COOH
22: X = CH, 4-COOH
23: X = N, 4-COOH

24

25: X = S
26: X = O

Agent	$EC_{50}(nM)^1$		
	RARα	RARβ	RARγ
1	5.0	1.5	0.5
2 0	NA^2	NA	NA
2 1	NA	NA	>1000
22 (AGN 190121)	598	2	10
2 3	NA	5	15
2 4	NA	355	930

$^1EC_{50}$ is the nanomoles of retinoid required for 50% activation by RARs after taking ATRA-induced activation by RARs as 100%. Its value for each receptor was determined by transfecting HeLa cells with reporter construct ERE-tk-CAT and one of the ER(ABC)-RAR-α(DEF), ER(ABC)-RAR-β(DEF) or ER(ABC)-RAR-γ(DEF) expression vectors. ^{2} NA indicates Not Active $(EC_{50}>10^4$ nM)

aromatic carboxylic acids (Table 2) [66]. Although these analogs have significant binding affinities to all three RARs (data not shown), they activate gene transcription primarily through RAR-β and γ. It can be seen that the *para*-linked benzoic acid is optimal for activity and that the pyridine carboxylic acid unit minimizes any residual activity at RAR-α. The cyclohexenyl ring along with the 7,8-double bond in ATRA was further modified to the reduced aromatic/heteroaromatic ring system containing acetylenic retinoids 27-38 that are all RAR-β, γ selective in transactivation assays (Table 3) [67,68]. From the data in Table 3, it can be inferred that lipophilic groups at either the C-1 or C-4 position of the retinoid skeleton seem to suffice for RAR activity although substitution at both positions leads to an increase in potency.

Tazarotene (6) (AGN 190168) [22,67,69], an RAR-β,γ selective acetylenic retinoid, was chosen for development as a topical agent for psoriasis and acne and is currently the only topical retinoid approved for the treatment of psoriasis. Its topical *in vivo* activity has been characterized by inhibition of 12-O-tetradecanoyl phorbol-13-acetate (TPA)-induced ODC (ornithine decarboxylase) activity in hairless mouse epidermis, and it is more potent than ATRA, acitretin, and adapalene (Table 4) [24,68]. ODC is a critical enzyme that is involved in polyamine biosynthesis [70] and its levels are elevated prior to a hyperproliferative response. In diseases that are characterized by hyperproliferation such as psoriasis, ODC inhibitors should be of therapeutic value. Tazarotene is an ethyl ester prodrug and these ester derivatives exhibit better

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Table 3. Transcriptional Activation Assay Data of Acetylenic Retinoids

27: W = X = Y = CH$_2$, Z = CH
28: W = C(CH$_3$)$_2$, Y = CH$_2$, Z = CH
29: X = W = CH$_2$, Y = C(CH$_3$)$_2$, Z = CH
30: W = Y = C(CH$_3$)$_2$, X = CH$_2$, Z = CH
31: W = Y = C(CH$_3$)$_2$, X = CH$_2$, Z = N
32: W = C(CH$_3$)$_2$, X = CH$_2$, Y = S, Z = CH

33: W = C(CH$_3$)$_2$, X = CH$_2$, Y = O, Z = CH
34: W = C(CH$_3$)$_2$, X = CH$_2$, Y = S, Z = N
35: W = C(CH$_3$)$_2$, X = CH$_2$, Y = O, Z = N
36: W = S, X = CH$_2$, Y = C(CH$_3$)$_2$, Z = CH
37: W = S, X = Y = C(CH$_3$)$_2$, Z = N
38: W = O, X = Y = C(CH$_3$)$_2$, Z = CH

Agent	EC$_{50}$(nM)[1]		
	RARα	RARβ	RARγ
1	5.0	1.5	0.5
27	>1000	>1000	>1000
28	186	1.4	5.2
29	915	6.1	11
30	62	0.7	0.4
31	61	0.3	0.4
32	169	3.6	40
33	169	0.6	8.1
34 (Tazarotenic acid)	587	1.7	69
35	>1000	15	81
36	>1000	4.24	12.8
37	>1000	12	13
38	791	1.7	2.7

[1]EC$_{50}$ is the nanomoles of retinoid required for 50% activation by RARs after taking RA-induced activation by RARs as 100%. Its value for each receptor was determined by transfecting HeLa cells with reporter construct ERE-tk-CAT and one of the ER(ABC)-RAR-α(DEF), ER(ABC)-RAR-β(DEF) or ER(ABC)-RAR-γ(DEF) expression vectors.

therapeutic indices in the skin relative to their free carboxylic acids (compare tazarotene and tazarotenic acid in Table 4) considering that both compounds produce equal topical irritation in hairless mice. Other features of tazarotene are also significant. The pyridine nitrogen was introduced to aid rapid metabolic hydrolysis to the free acid [71], thus preventing the accumulation of the lipophilic ester in fatty tissue and avoiding the extended elimination half-life that is characteristic of retinoid esters such as etretinate [72]. The sulfur atom in the left-hand lipophilic portion was introduced to enable facile systemic oxidation to inactive

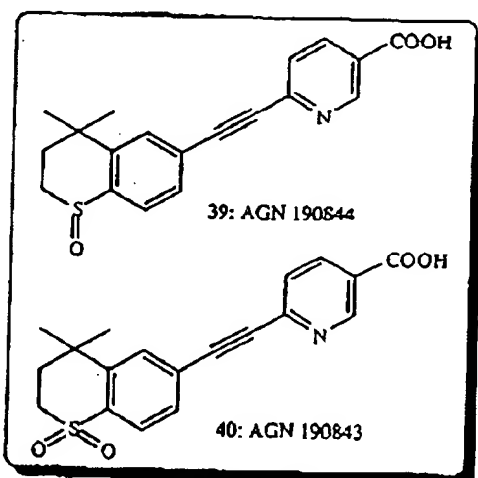sulfoxide 39 (AGN 190844) and sulfone 40 (AGN 190843) forms [73].

(ii) RAR-α Specific Agonists

The internal amide series of compounds exemplified by Am 580 (41) and Am 80 (42) [74] were synthesized by Shudo et al., and the RAR-α selectivity of these compounds was demonstrated subsequently [75]. The introduction of the hydrophilic amide linkage would be expected to destabilize the interaction at all three RARs but decreased binding is observed only at RAR-β and

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Table 4. ODC Inhibitory Activities of Therapeutic Retinoids In Hairless Mouse

	IC_{50}(nanomoles)[1]
1 (ATRA)	1.4
34 (Tazarotenic acid)	7.5
3 (13-cis RA)	2.7
4 (Etretinate)	27
5 (Acitretin)	5.7
6 (Tazarotene)	0.85
7 (Adapalene)	35
39 (AGN 190844)	>300
40 (AGN 190843)	>300

[1] IC_{50} values determined in dose response studies of hairless mice. Retinoid is applied 1 hr before TPA, and ODC activity is measured in mouse epidermis 4 hours later.

Interestingly, the affinities of **43** and **44** for RAR-α are slightly higher than of even the natural hormone ATRA.

41: Am 580

42: Am 80

39: AGN 190844

40: AGN 190843

43: X = H. AGN 193835
44. X = F. AGN 193836

Table 5. Binding Affinities K_d(nM) of Amide-linked Retinoids

	K_d(nM)[1]		
	RARα	RARβ	RARγ
1 ATRA	15±1.7	13±2.5	18±1
10 TTNPB	72±37	5±2	26±20
41 AM 580	36±1.5	1361±321	3824±2644
43 AGN 193835	4.4±2.3	3037±859	>30000
44 AGN 193836	8.4±1.3	17374±6347	>30000

[1] K_d values (mean ± SEM of triplicate determinations) determined via competition of [3H]-(all-trans)-retinoic acid (5 nM) binding with unlabeled test retinoid at baculovirus-expressed RARs and application of the equation of Cheng-Prusof [78].

RAR-γ. The ligand-binding residues identified in the crystal structure of RAR-γ bound to ATRA [50] include an alanine in helix-3 of the ligand-binding domain and this is replaced by a serine in RAR-α. This serine can interact with the amide linkage of Am 580/Am 80 by hydrogen bonding, thereby providing a rationale for the observed RAR-α selectivity of these analogs [76].

Highly RAR-α specific amide derivatives such as **43** (AGN 193835) and **44** (AGN 193836) [77] were subsequently synthesized. As seen in Table 5, introduction of the fluoro group *ortho* to the carboxylic acid increases the binding affinity at RAR-α without effecting any binding at RAR-β and γ. Introduction of a second fluoro group enhances the α/β selectivity to 2000 fold.

45

46

47

(iii) RAR-β Selective Agonists

RAR-β selective agonists that function as antagonists at RAR-α and γ were reported but their chemical structures were not disclosed [79]. The 2-thienyl substituted dihydronaphthalene retinoids 45-47 also activate gene transcription only through RAR-β [80] while binding to all RARs (Table 6). The tether region is varied from the acetylenic unit in 45 to the amide linkage in 46 and the ester linkage in 47. In compound 46, although the amide linkage selects for binding affinity at RAR-α, the 2-thienyl group determines the transcriptional activity resulting in an RARβ-specific transactivator. These analogs are partial agonists at RAR-β and antagonists at RAR-α and γ.
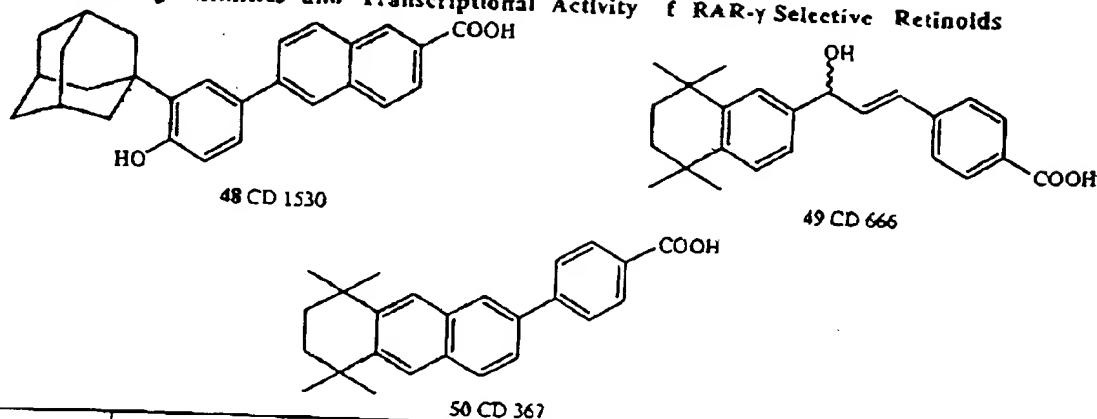
(iv) RAR-γ Selective Agonists

The analogs 48 and 49 [81], showed good RAR-γ selectivity in terms of binding and moderate RAR-γ selectivity in the transcriptional assay as seen in Table 7. The previously discussed retinoid 19 is moderately RAR-γ selective in binding and this selectivity is retained in transactivation assays. The compounds 51-53 were also found to be RAR-γ selective [82,83] and their receptor binding and transactivation data are summarized in Table 8 [84].

Table 6.   **Binding Affinities and Transactivation Data for RAR-β Selectives**

	K_d (nM)[1]			EC_{50} (nM)[2]			% Efficacy[3]		
	α	β	γ	α	β	γ	α	β	γ
1	15±2	13±3	18±1	459	87	20			
45	129±6	20±2	104±30	NA[4]	25±4	NA		32	
46	94±15	996±148	>10000	NA	115±37	NA		61	
47	303±32	189±41	1490±106	NA	26±2	NA		47	

[1]K_d values were determined via competition of [³H]-ATRA (5nM) binding with unlabeled test retinoid at baculovirus expressed RARs and application of the equation of Cheng and Prusoff [78]. [2]Transactivation assays were performed in CV-1 cells cotransfected with the reporter plasmid MTV-4(RSO)-LUC and an expression vector of the indicated retinoid receptor. [3]% Efficacy = normalized to the maximum ATRA response and reported as an average of three experiments. [4]NA indicates Not Active.
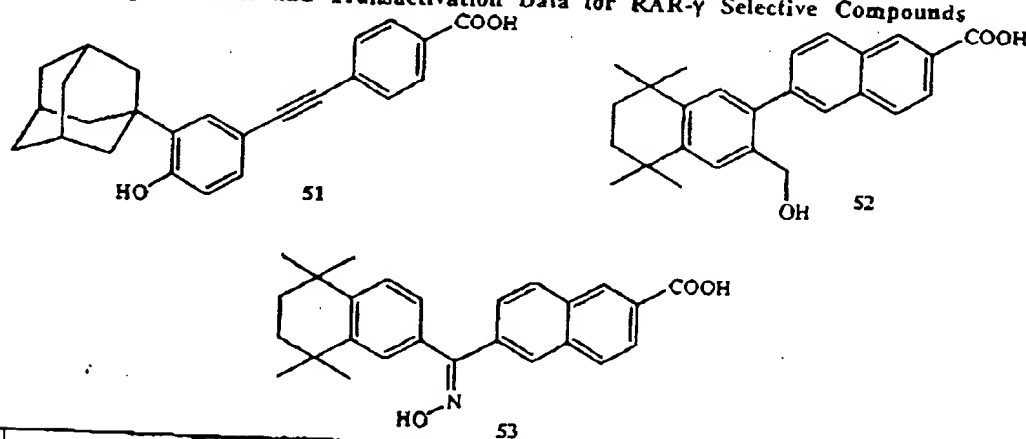
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Table 7.   Binding Affinities and Transcriptional Activity f RAR-γ Selective Retinoids

48 CD 1530

49 CD 666

50 CD 367

Compd	K_d (nM)[1]			AC_{50} (nM) [2]		
	RAR-α	RAR-β	RAR-γ	RAR-α	RAR-β	RAR-γ
1 (ATRA)	15.5	4.5	3.0	2.12	3.62	2.46
19	6500	2480	77	140	28.4	7.3
48 (CD1530)	2750	1500	150	37.5	28.5	1.8
49 (CD 666)	2240	2300	68	149	50.8	1.4

[1]Values were obtained in saturation experiments with reference compound CD 367 (compound 50) and in competition experiments with other synthetic retinoids and are the mean of two or three determinations. Assay performed on nuclear extracts of COS-7 cells transfected with pSG5-derived expression vectors for the three RARs. [2]Values of transcriptional activation by RA and synthetic retinoids are reported and are the mean of two to six determinations. HeLa cells cotransfected with expression vectors for human RAR-α,β or γ with TREpal-u-CAT reporter plasmid were used in assay.
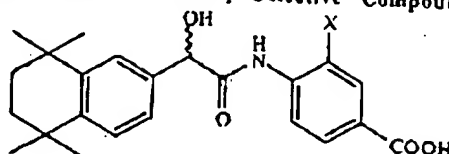
Table 8.   Binding Affinities and Transactivation Data for RAR-γ Selective Compounds

51

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53

	K_d (nM)[1]			EC_{50} (nM)[2]			% Efficacy[3]		
	RAR-α	RAR-α	RAR-γ	RAR-α	RAR-β	RAR-γ	RAR-α	RAR-β	RAR-γ
1	2.8	2.1	0.6	45	5	2	100	86	87
51	>1000	>1000	15	>1000	2800	340	1	15	67
52	>1000	>1000	22	>1000	>1000	170	8	39	170
53	>1000	>1000	20	>1000	1600	22	13	46	84

[1]K_d values were determined via competition of [3H]-ATRA binding with unlabeled test retinoid. [2]Transactivation assays were performed in CV-1 cells using the TREpal-u-CAT reporter construct. [3]% activity at 1000 nM relative to ATRA.

Table 9.   Receptor Transactivation Ratios of RAR-γ Selective Compounds

54-57 (54: BMS 188961)

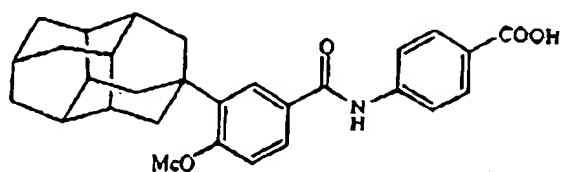| Compd	Substitution X	Transactivation ratio[1]		
		RAR-α	RAR-β	RAR-γ
1 (ATRA)		1	1	1
54 (BMS 188961)	F	NA[2]	WA[3]	3.8
55	Cl	NA	NA	13.3
56	OH	NA	NA	40
57	Me	NA	NA	67

[1] The ratio of the EC$_{50}$ of the test compound at a receptor to that of ATRA at the same receptor. [2] NA indicates Not Active. [3] WA indicates Weakly Active.

A third series of α-hydroxyacetamide-linked retinoids 54-57 [85,86] are shown in Table 9. These compounds have a common 3-atom tether and also a common tetramethyl tetralin for the hydrophobic unit. Compound 54 (BMS 188961) which has a fluoro substituent on the benzoic acid is the most potent RAR-γ agonist, although it has partial agonist activity at RAR-β (35% of the maximum transactivation at a 1μM dose). The chloro-substituted analog 55 is quite potent and is the most selective RAR-γ agonist.
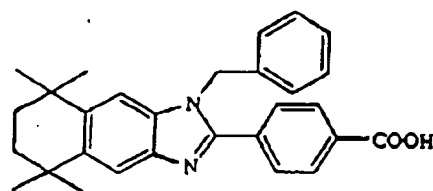
b. RAR Antagonists

A variety of RAR antagonists have been reported in the literature. The first examples were compounds 58 and 59 [87], both of which antagonize ATRA-induced HL-60 cell differentiation [88] at concentrations roughly 100-fold in excess of the ATRA concentration used. A series of N-substituted pyrrole and pyrazole ring containing compounds exemplified by 60 and 61 [89] were also found to display retinoid antagonist activity in the HL-60 cell differentiation assay and were found to be the most potent antagonists in their class with IC$_{50}$ values in the nanomolar range. The anthracenyl retinoid 62 is inactive in the RAR transcriptional assay and antagonizes transcription induced by ATRA effectively [90,91]. Mechanistic studies showed that it functions as an antagonist by competing for the receptor binding sites with ATRA.
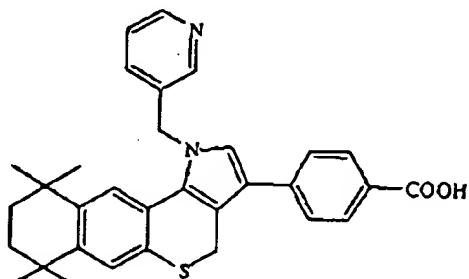
Systematic structure-activity relationship (SAR) studies demonstrated that introduction of a toluyl group at the C-1 position of the dihydronaphthalenyl acetylenic skeleton imparts RAR antagonistic property to compound 63 [92]. While compound 63 (AGN 193109) has very high binding affinities (K$_d$ = 2-3nM) to the RARs, it has absolutely no activity in RAR transcriptional assays. Furthermore, it inhibited the gene transcription activity induced by ATRA in a dose-responsive manner (Table 10). At a concentration 10-fold in excess over ATRA, compound 63 was able to totally abrogate transactivation at all three RARs. The effect of substituents on the C-1 aryl ring was examined and the binding affinities for the compounds 64-77 are shown in Table 11. With the exception of compounds 64, 65 and 71 (AGN 193840), which are partial agonists at RAR-β, all the other compounds were totally inactive in activating gene transcription at the RARs and functioned as antagonists of ATRA-induced RAR transactivation. The data in Table 11 points to three main observations. Firstly, the *meta*-substituent on the C-1 phenyl ring is detrimental to binding at the RARs. Secondly, the size rather than the polarity of the *para*-substituent appears to be of relevance to RAR binding since the *para*-chloro, trifluoromethyl, ethyl and methoxy substituted analogs have similar binding affinities. Thirdly, the RAR-α and RAR-γ receptors appear to be more sensitive to changes in substituents on the C-1 phenyl ring.
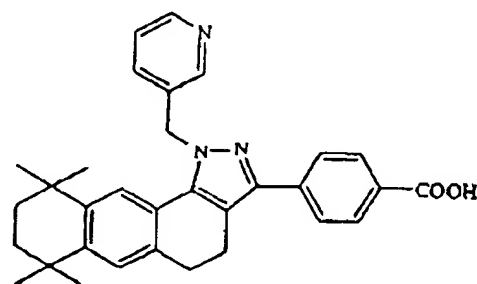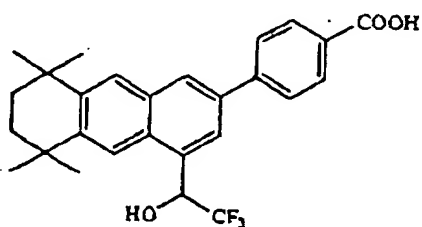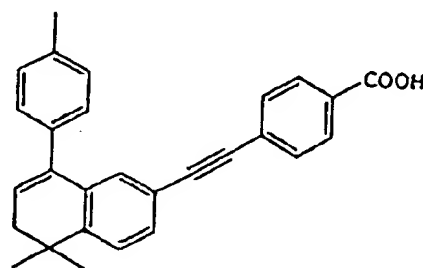
58

59

60

61

62

63: AGN 193109

Table 10.   RAR Panantagonism of 63

	K_d (nM)[1]			EC_{50} (nM)[2]			IC_{50}[3]		
	α	β	γ	α	β	γ	α	β	γ
1 (ATRA)	9	12	19	7	1	0.7			
63 (AGN 193109)	2	2	3	NA[4]	NA	NA	9±1	24±3	5±1

[1] K_d values were determined via competition of [³H]-ATRA (5nM) binding with unlabeled test retinoid at baculovirus expressed RARs and application of the equation of Cheng and Prusof [78]. [2] Transactivation assays were performed in CV-1 cells cotransfected with the reporter plasmid ΔMTV-TREp-LUC and an expression vector of the indicated retinoid receptor. [3] IC_{50} values represent the mean ± S.E. of at least three independent assays using antagonism of a 10^{-8} M dose of ATRA. [4] NA indicates Not Active.

Table 11.   Binding Affinities[1] of RAR Antagonists of the Dihydronaphthalene Series

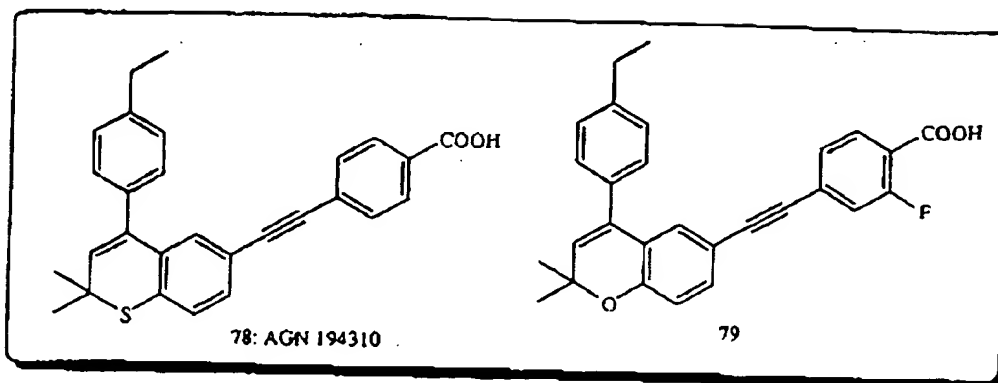| Compound	Ar	K_d(nM)		
		RAR-α	RAR-β	RAR-γ
ATRA 1		15±2	13±3	18±1
64	C_6H_5-	147±27	33±6	42±9
65	2-Me-C_6H_4-	103±17	29±8	35±9
66	3-Me-C_6H_4-	276±175	51±22	67±30
63 (AGN 193109)	4-Me-C_6H_4-	16±5	7±3	7±1
67	3,5-DiMe-C_6H_4-	1051±802	274±45	408±146
68	4-Et-C_6H_4-	40±16	10±2	19±8
69	4-*i*-Pr-C_6H_4-	90±10	11±4	171±51
70	4-*t*-Bu-C_6H_4-	178±81	21±4	94±63
71 (AGN 193840)	4-F-C_6H_4-	85±45	52±30	82±35
72	4-Cl-C_6H_4-	58±9	18±2	27±8
73	4-CF_3-C_6H_4-	44±7	29±5	57±28
74	3-OH-C_6H_4-	919±250	162±85	254±118
75	4-OH-C_6H_4-	229±48	72±21	98±24
76	4-OMe-C_6H_4-	60±29	16±1	36±7
77	4-CN-C_6H_4-	38±5	14±3	51±15

[1]K_d values were determined via competition binding experiments using [^{3}H]-ATRA (5 nM) with unlabeled test retinoid at baculovirus-expressed RARs.

The dihydronaphthalene unit in the compounds 63-77 as well as the pendant aromatic groups were changed in a variety of analogs and of these, compounds 78 (AGN 194310) and 79 were found to be the most effective RAR antagonists [14]. The RAR antagonists 63, 78 and 79 were evaluated for their ability to inhibit TTNPB-induced topical toxicity and these results are summarized in Table 12. It can be seen that when these compounds are used in an 8-fold excess over TTNPB, they are able to completely abolish the retinoid agonist induced toxicity.

Table 12. At Various Antagonist-TTNPB Ratios[1], Percent Inhibition[2] of TTNPB Induced Cutaneous Toxicity

	Ratio		
	0.5	2.0	8.0
63 (AGN 193109)	72	85	98
78 (AGN 194310)	79	84	94
79	71	86	92

[1] Ratio = [antagonist]/[TTNPB]. [2] Determined using the cutaneous toxicity scores for TTNPB with vehicle control and in combination with antagonist.
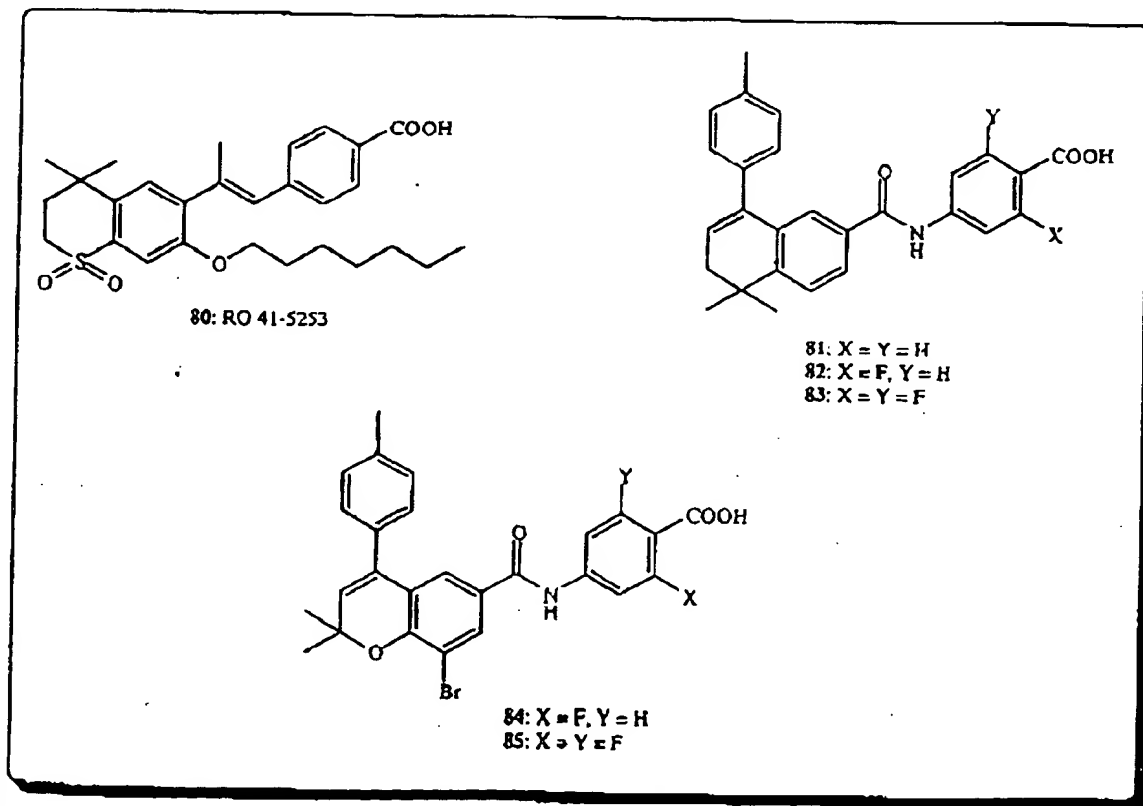
78: AGN 194310

79

(i) RAR-subtype selective antagonists

There are only two reports on RAR subtype selective antagonists. The stilbene analog **80** [93,94] is an RAR-α selective antagonist. However it has to be used in 1000-fold molar excess in order to eliminate the transcriptional response of ATRA. The second series of RAR-α antagonists **81-85** were developed by incorporating the structural features necessary for RAR antagonism in compound **63** [95] and features responsible for RAR-α binding, namely an amide linkage in the tether region. Of these, compounds **84** and **85** were the most effective antagonists at RAR-α,

abolishing the response of ATRA at RAR-α when present in one-tenth or equimolar concentrations, respectively, to that of ATRA.

(ii) Neutral antagonists vs Inverse agonists

The concept of inverse agonism originated in the G-protein-coupled receptor area, where an inverse agonist repressed the basal activity of an unliganded receptor [96,97]. The proposed model for inverse agonism envisages an equilibrium between spontaneously active and inactive receptors in the absence of a ligand [98]. Inverse agonists shift this

80: RO 41-5253

81: X = Y = H
82: X = F, Y = H
83: X = Y = F

84: X = F, Y = H
85: X = Y = F

equilibrium towards inactive receptors whereas agonists shift it towards active receptors. A neutral antagonist has no effect on this equilibrium, but competitively inhibits both agonist as well as inverse agonist induced changes in equilibrium. A general model for nuclear receptors proposes an equilibrium between the unliganded receptor and co-activator and co-repressor molecules [29]. Receptor agonists shift the equilibrium to a co-activator bound receptor thereby increasing the level of gene transcription, while inverse agonists lead to a co-repressor bound receptor resulting in a decrease in basal transcriptional activity [15,16].

The diaryl acetylenic retinoid antagonists 63, 64, 71, 72 and 73 were evaluated for their ability to inhibit the basal transcription of a chimaeric RARγ-VP-16 [15]. VP-16 is the constitutively active transactivation domain of the herpes simplex virus (HSV) and confers a high level of basal activity to RARγ-VP-16. AGN 193109 (63), compound 72 and compound 73 function as inverse agonists by repressing the strong transcriptional activity of the VP-16 transactivation domain. In contrast, although compounds 64 and 71 (AGN 193840) are potent and effective antagonists of ATRA at RAR-γ, they do not transrepress RARγ-VP-16. These compounds therefore are neutral antagonists. The inverse agonists have bulky substituents such as the methyl, trifluoromethyl and chloro group at the 4-position of the phenyl ring whereas the neutral antagonists have relatively smaller hydrogen and fluorine atoms at the same position. The neutral antagonists can antagonize both the transactivation effects of agonists and the transrepression effects of inverse agonists in a dose-responsive manner [15,16].

The inverse agonist AGN 193109 (63) was found to inhibit the skin toxicity, splenomegaly and weight loss induced in mice by topical and oral treatment of TTNPB (10) [99] in regimens involving co-treatment as well as pretreatment. Retinoid antagonists thus have the potential to alleviate clinical retinoid agonist-induced toxicities. AGN 194310 (78), a potent antagonist, is presently in pre-clinical development as a topical antidote for systemic retinoid induced toxicity and for the topical treatment of inflammatory skin conditions.

MRP-8 (migratory inhibition related factor) is a marker of abnormal differentiation that is present in high levels in psoriatic skin, but is absent in normal human skin. In cultured human keratinocytes, RAR panagonists like TTNPB and RAR-β,γ selective

agonists such as tazarotene inhibit the expression of MRP-8 [22]. Interestingly, the inverse agonist AGN 193109 (63) was also found to inhibit the expression of MRP-8 in cultured human keratinocytes, whereas the neutral antagonist 71 (AGN 193840) had no effect [15]. Simultaneous treatment with the inverse agonist and a panagonist such as TTNPB however, results in a mutual repression of the inhibitory activity. The inhibitory effect is restored when either compound is present in significant excess [16]. This study indicates distinct yet overlapping mechanisms for the MRP-8 inhibitory ability of the two classes and implicates RAR-γ as the common receptor. Support for the involvement of RAR-γ stems from the predominance of RAR-γ subtype in cultured human keratinocytes and the fact that the RAR-α selective agonist AGN 193836 (44) has no effect on MRP-8 expression in cultured human keratinocytes. MRP-8 regulation can be used as a predictive assay for the efficacy of retinoids in psoriasis. Since AGN 193109 (63) inhibits the expression of MRP-8, there is potential for the use of RAR inverse agonists in the treatment of psoriasis.

The RAR panagonist TTNPB (10) suppresses the proliferation of the human papilloma virus-immortalized ectocervical cell line ECE16-1 with a concomitant increase in RAR-β mRNA levels [100]. The inverse agonist AGN 193109 (63) was found to inhibit both these effects induced by TTNPB. However, it did not possess any intrinsic anti-proliferative activity in these cells. In the same cells, while TTNPB inhibited MRP-8 expression, AGN 193109 induced MRP-8 expression. These and earlier observations indicate that the agonist-inverse agonist relationship can be gene and cell type specific [16].

c. AP-1 Antagonism at RAR

Retinoids are able to repress gene expression by antagonizing the activity of other transcription factors such as AP-1 [68,101] and NF-IL6 (nuclear factor-interleukin 6) [102]. Through these negative regulatory pathways, metalloproteinases, cytokines, and certain other genes involved in cell proliferation and inflammation, are repressed by retinoids. AP-1 is an oncoprotein that is involved in hyperproliferative and inflammatory responses in diseases such as psoriasis and arthritis and may have a function in tumor metastases [101,103-105]. Retinoids which separate inhibition of AP-1 activity from receptor transactivation, have been previously reported [68,106], but so far no clear structure-activity relationships have emerged for this functional separation.

d. RXR Agonists

The first report on a series of compounds that activated the RXR-RXR homodimers was published in 1992 [60]. The subsequent synthesis of radiolabeled 9-*cis*-RA led to the rapid development of numerous additional RXR ligands [107]. The isosteres for the 9-*cis*-double bond of 9-*cis*-RA 2 that have been successfully incorporated so far fall into two categories. The first category consists of single atom bridged compounds such as the sp³ carbon bridged arotinoids SR 11237 (86) [60] and the more recent LG 100268 (9) [108], the sp² carbon linked Targretin (LG 100069: 8) [109] and the sulfur atom bridged retinoid 87 (AGN 192849) [110]. The second category consists of compounds that have rings of various sizes with the formal 9-cis double bond incorporated into the ring exemplified by the potent RXR agonist 88 [111]. More recently, analogs of 9-*cis*-retinoic acid such as 89 that have a fluoro group at the 10-position and a tetrahydroquinoline left hand unit have been synthesized and shown to be potent RXR agonists [112].

(i) The α-Methyl Effect

TTNPB, a stilbene derivative, is a potent RAR agonist with absolutely no affinity to or activity at the RXR receptors. When a methyl group is introduced on the tetralin ring at the 3-position, however, the resulting compound 90 is endowed with significant gene transcriptional activity at RXR-α, at the expense of activity at the RARs [113]. In order to rule out the possibility that the RXR activity could be attributed to a metabolite resulting from the oxidation of the 3-methyl group, other groups that are inert to oxidative metabolism such as the 3-bromo and 3-chloro groups were introduced. The resulting compounds are structurally similar to 3-methyl TTNPB (90). They had similar transcriptional profiles to that of compound 90 indicating that the conformational change effected by the α-substituent enabled them to activate gene transcription at RXR-α (Table 13). As can be seen in Table 13, the absence of the C-9 methyl group also leads to a decrease in transcriptional activity at RXR-α. Another report probed the effect of introducing various lower alkyl groups at the 3- position of TTNPB [107]. Increasing the size of the group at the 3-position from methyl to ethyl led to a considerable drop in binding affinity to RXR-α (data not shown). Also, the effect of substituting the benzoic acid moiety in 3-methyl-TTNPB with heteroaromatic carboxylic acids that also varied in the position of the carboxylic acid was studied [114]. The orientation of the carboxylic acid was found to have a dramatic influence on the receptor profiles of these compounds (data not shown). From these results, it was proposed that a twist about the bond between C-2 of the tetralin and the C-9 carbon is responsible for the increased RXR and decreased RAR potency.
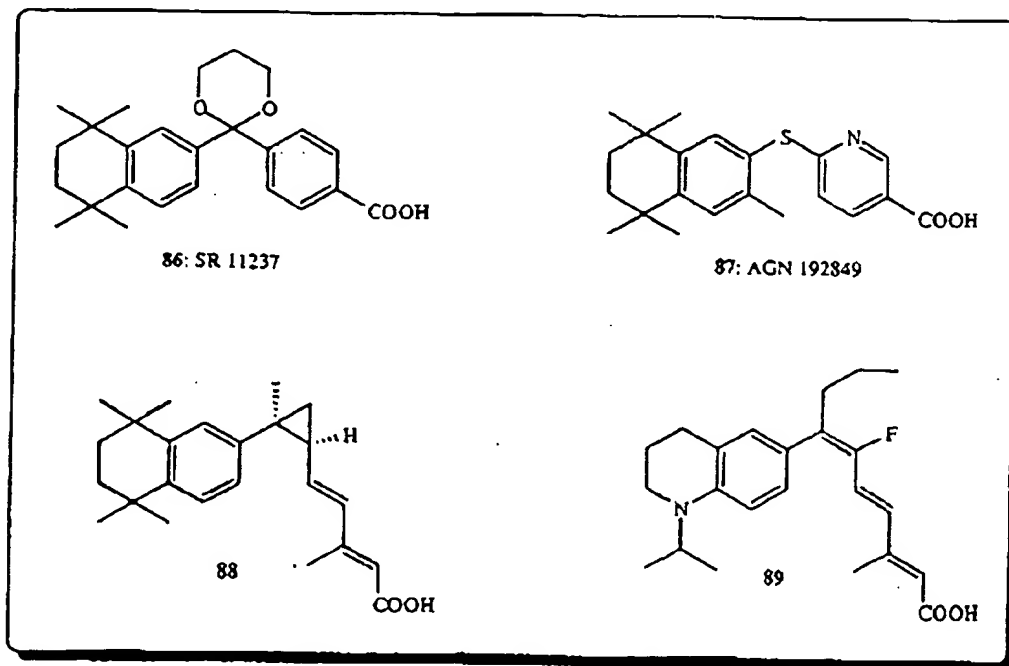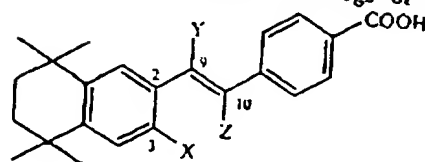

86: SR 11237

87: AGN 192849

88

89

Ligands of Retinoid Receptors

Current Pharmaceutical Design, 2000, Vol. 6, No. 1   41

Table 13. Transcriptional Assay Data for 3-Substituted Analogs of TTNPB

Compd	Stilbene substitution			EC$_{50}$ (nM)			
	X	Y	Z	RAR-α	RAR-β	RAR-γ	RXR-α
ATRA 1				5.0	1.5	0.5	NA[1]
9-cis-RA 2				102	3.3	6.0	13.0
10 (TTNPB)	H	Me	H	21.0	4.0	2.4	NA
90	Me	Me	H	4580	74	152	385
91	Cl	Me	H	>1000	21.0	77.0	275
92	Br	Me	H	989	21.0	91.0	298
93	Et	Me	H	NA	961	195	2220
94 (Z-olefin)	Me	Me	H	NA	NA	NA	>6400
95	Me	H	H	15	0.4	1.4	NA
96	H	H	H	11.0	0.4	0.4	NA
97	H	H	Me	24.0	0.5	0.4	NA

[1] NA indicates Not Active

The α-methyl effect was also observed in the sulfur-bridged retinoids of which compound 87 is an example. This was expected since the relative orientation of the two aromatic rings would be twisted as in 3-methyl TTNPB upon introduction of the 3-methyl substituent. The diaryl sulfides 87 and 98-100 and the sulfone and sulfoxide derivatives 101-102 were made (Table 14) to investigate this hypothesis [110]. The desmethyl compound 98 was neither RXR specific nor potent at RXR-α while the α-methyl substituted analog, 87, was a moderately potent RXR-specific analog. The sulfone and sulfoxide forms, 101 and 102, have diminished transcriptional activity suggesting that these disulfide analogs can be potentially deactivated in vivo by oxidative metabolism.
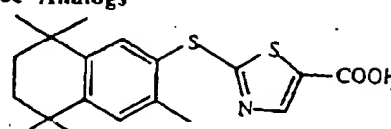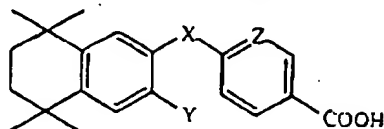
The sp2 carbon linked retinoid Targretin (8) and the sp3 carbon bridged retinoid LG 100268 (9) are both examples of RXR agonists with α-methyl groups. As seen in Table 15, LG 100268 is one of the most potent RXR agonists in the one atom-bridged class of compounds [108]. The sp2 carbon atom bridged oximes 103-107 (Table 16) [115] also exhibit the α-methyl effect. In this series, the trans stereochemistry of the oxime is very important for potent RXR agonism. The free oximes and the oxime ethers are equally active. There is a substantial drop in binding affinities and potencies at the RXRs for oxime ethers with alkyl groups larger than the n-propyl group (data not shown). Like agonists for PPAR-γ, these compounds have been shown to be potent inducers of differentiation of 3T3-L1 pre-adipocytes [115].

(ii) 9-cis-locked compounds

Since 9-cis RA binds and activates both RARs and RXRs it is not a suitable compound for investigating RXR biology. Also, the isomerization of 9-cis RA to ATRA in experimental systems further complicates interpretation of results. It was hypothesized that 9-cis-ring locked compounds would prevent this isomerism and might also result

Table 14. Transactivation Assay Data for Diarylsulfide Analogs

87: X = CH₃, Y = S, Z = N
98: X = H, Y = S, Z = CH
99: X = CH₃, Y = S, Z = CH
101: X = CH₃, Y = SO, Z = CH
102: X = CH₃, Y = SO₂, Z = CH

100

Compound	EC₅₀ (nM) Efficacy (% ATRA)					
	RARα	RARβ	RARγ	RXRα	RXRβ	RXRγ
ATRA 1	350	80	10	900	1400	1100
9-cis-RA 2	191	50	45	100	200	140
87 (AGN 192849)	NA¹ (1)	NA (4)	NA (0)	54 (91)	57 (100)	42 (85)
98	NA (3)	570 (66)	340 (37)	770 (51)	1600 (80)	1600 (75)
99	NA (3)	NA (4)	NA (6)	280 (57	200 (74)	280 (112)
100	NA (1)	NA (0)	NA (7)	2300 (85)	1300 (117)	1900 (70)
101	NA (5)	NA (4)	NA (3)	NA (17)	3000 (29)	1600 (28)
102	NA (2)	NA (6)	NA (0)	2800 (55)	2600 (52)	2600 (45)

¹NA indicates Not Active

in compounds that are potent and specific at the RXRs. The cyclopropyl ring locked tetralin dienoic acids 108-113 were synthesized for this purpose and their receptor data are summarized in Table 17 [116]. The strategy was successful since compound 108 is a highly potent and completely specific RXR agonist. Other groups have also reported on such ring-locked compounds [117]. A key observation for these compounds is that unlike in the 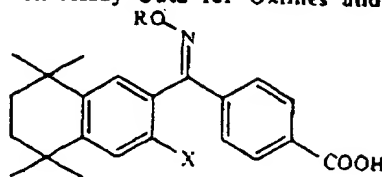stilbene and diaryl sulfide series, the introduction of a methyl group at the 3-position of the tetralin ring is detrimental to both the binding and transactivation at the RXRs [116]. Another important observation from these series is that the optically pure dextro rotatory isomer of compound 108, namely compound 88, is nearly 100 times more potent than its levo rotatory counterpart [111]. The furan and benzene ring locked 9-cis analogs, 114 and 115 [118], are also potent and selective RXR agonists (data not shown).

Table 15. Binding and Transactivation Assay Data for LG 100268 and LG 100069

	Binding affinity¹ Kᵢ (nM)			EC₅₀ (nM) ²		
	RXRα	RXRβ	RXRγ	RXRα	RXRβ	RXRγ
8 (LG 100069)	36	21	29	28	25	20
9 (LG 100268)	3	3	3	4	3	4

¹Competitive binding assay using baculovirus expressed RXRs and the Kᵢ values are mean ± SEM of an average of three experiments. ²Cotransfection assays in CV-1 cells transfected with an expression vector for each of the RXRs.
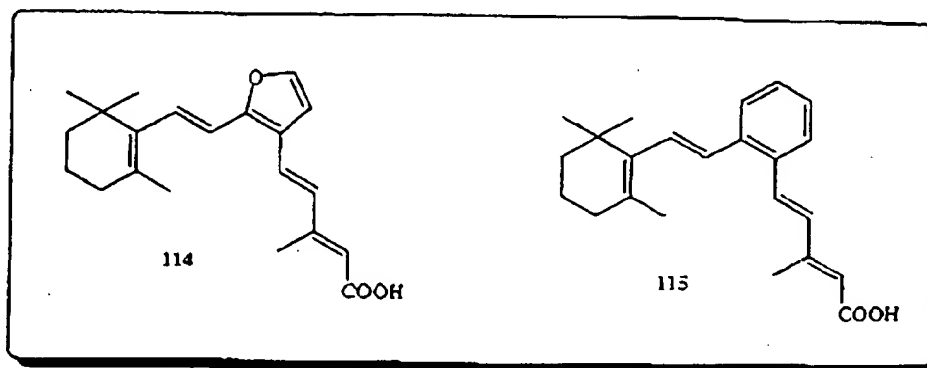
Table 16. Binding and Transactivation Assay Data for Oximes and Oxime Ethers

103-107

	X	R	Binding affinity[1] K_i (nM)			EC_{50} (nM)[2]		
			RXRα	RXRβ	RXRγ	RXRα	RXRβ	RXRγ
103	Me	H	6	5	5	7	14	7
104	Me	Me	9	8	8	5	10	4
105	Br	H	12	6	57	27	32	26
106	H	H	4234	3363	5051	2955	$>10^4$	2894
107	H	Me	>1000	363	558	253	207	206

[1]Competitive binding assay using baculovirus expressed RXRs. [2]Cotransfection assays in CV-1 cells transfected with an expression vector for each of the RXRs.

114

115

e. RXR Antagonists

The tetralin based trienoic acid **116** (LG 100754) was the first RXR homodimer antagonist to be identified [119]. As is evident from Table 18, two effects are observed as the alkoxy group at the 3-position of the tetralin is varied from methoxy through ethoxy to *n*-propyloxy. Firstly, binding specificity to RXRs is increased, while the high binding affinities to the RXRs are retained. Secondly, transactivation at the RXRs decreases dramatically. In functional antagonism assays, compound **116** inhibited the transcriptional response of compound **8** (LG 1069) in CV-1 cells in a dose dependent manner with IC_{50}s of 16, 28 and 19 nM at RXRs-α, β and γ, respectively. At equimolar concentrations of the agonist **8** and the

antagonist **116**, RXR transactivation was inhibited by approximately 50%.

4. RETINOID THERAPEUTICS: INDICATIONS, MECHANISMS, AND FUTURE USES

a. Vitamin A Supplementation

Vitamin A-deficiency is a major cause of mortality and morbidity in infant and child populations in underdeveloped countries. It is estimated that upwards of 250,000 children go blind due to Vitamin A deficiency each year [120]. An additional consequence of Vitamin A deficiency

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Table 17. Binding and Transactivation Data of Cyclopropyldienoic Acids

108:   R = H, 13E, racemic at 9, 10
88:   R = H, 13E, optically pure dextro rotatory isomer
109:   R = H, 13E, optically pure levo rotatory isomer
110:   R = Me, 13E
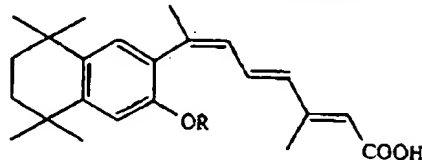111:   R = Me, 13Z

112: 13E; 113: 13Z

Number/name		RAR-α	RAR-β	RAR-γ	RXR-α	RXR-β	RXR-γ
ATRA 1	K_d	15	13	18	>1000	>1000	350
	EC_{50}	7	1	0.7	900	1400	1100
2	K_d	11	7	22	9	.11	16
	EC_{50}	191	50	45	250	200	140
108	K_d	>1000	>1000	>1000	1.5	2.5	1.8
	EC_{50}	>1000	>1000	>1000	1.5	2.0	1.0
88	K_d	>1000	>1000	>1000	0.5	3.4	2.5
	EC_{50}	>1000	>1000	>1000	0.5	0.7	0.6
109	K_d	>1000	>1000	>1000	60	210	75
	EC_{50}	>1000	>1000	60	27	26	25
110	K_d	>1000	>1000	>1000	71	56	42
	EC_{50}	>1000	>1000	>1000	130	100	71
111	K_d	>1000	>1000	>1000	>1000	>1000	>1000
	EC_{50}	>1000	>1000	>1000	>1000	>1000	>1000
112	K_d	556	>1000	>1000	>1000	>1000	>1000
	EC_{50}	>1000	340	200	>1000	>1000	>1000
113	K_d	>1000	>1000	>1000	>1000	>1000	>1000
	EC_{50}	>1000	>1000	>1000	>1000	>1000	>1000

is increased susceptibility to childhood infectious disease, including measles, diarrhea, and respiratory infection [120,121]. Vitamin A supplementation is a partially effective stop gap measure for affected children. Other forms of clinical malnutrition have an adverse impact on Vitamin A absorption and distribution, and therefore successful programs to eliminate the symptoms of Vitamin A deficiency have required an integrated approach to both nutrition and economic development [120].

The sequence of events preceding blindness due to Vitamin A deficiency includes xerophthalmia, in which the corneal epithelium is unable to differentiate normally and becomes dry, scaly and susceptible to infection, and keratomalacia, in which the underlying stroma begins to liquefy and

Table 18. Binding and Transactivation Data of RXR Antagonist

117: R = Me
118: R = Et
116: R = n-propyl

Number/name		RAR-α	RAR-β	RAR-γ	RXR-α	RXR-β	RXR-γ
	K_d (nM)	306	400	437	2	2	4
117	EC_{50}(nM)	40	8	17	5	8	9
	% Eff.	35%	54%	66%	51%	102%	68%
	K_d(nM)	2582	3115	4206	4	12	17
118	EC_{50}(nM)	-	31	121	8	9	7
	% Eff.	14%	27%	42%	17%	31%	25%
	K_d(nM)	1791	2587	6094	8	9	14
116(LG 100754)	EC_{50}(nM)	-	-	192	-	-	-
	% Eff.	4%	10%	27%	2%	13%	4%

become necrotic [122]. The reversal of normal corneal differentiation is typical of the effect of Vitamin A deficiency in many other epithelia. In the trachea and urinary tract, for example, as in cornea, differentiated epithelia are converted to a less specialized stratified squamous epithelium with loss of specialized function [123]. Xerophthalmia is readily reversible with Vitamin A supplementation, but if infection or corneal ulceration and scarring have taken place, vision can be permanently impaired or lost. Vitamin A deficiency also has a significant effect on immune function, and animal studies have demonstrated that retinoids affect both lymphocyte development and antibody production [124]. Both of these activities of Vitamin A, control of normal epithelial differentiation and immune function, underlie retinoid therapeutic effects in dermatology and cancer.

Retinoid action in several conditions, including acne [125], psoriasis [126,127], chemoprevention of cancer [128], and treatment of inherited diseases of the skin, such as the ichthyoses [129], depend on regulation of epithelial differentiation. In these cases, there is no direct indication that Vitamin A deficiency itself is causative in development of the disease, although Vitamin A-deficiency in

experimental animals has been clearly shown to increase susceptibility to carcinogenesis [123].

b. Retinoid Pharmacology in Skin

Studies of retinoid action on epidermis in both humans and animals provide some insight into retinoid therapeutics in dermatology. Retinoids can have either proliferative or anti-proliferative effects in epidermis depending on the initial state of the tissue. In normal skin, retinoids are trophic or hyperproliferative, leading to increased cell turnover and increased thickness of the epidermal living layer [130]. An important step in retinoid-induced hyperplasia appears to be induction of heparin-binding EGF-like growth factor (HB-EGF) in the granular layer, one of the differentiated or suprabasal layers of epidermis. Expression of a dominant negative RAR in the granular layer blocks both retinoid-induced hyperplasia and HB-EGF expression [131]. Experimental models of retinoid anti-hyperproliferative effects are also well established. For example, retinoids block several actions of TPA, including tumor promotion, induction of the polyamine synthetic enzyme ornithine decarboxylase (ODC), and an initial phase

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of TPA-induced hyperproliferation in normal skin [132,133].

Both the hyperproliferative and anti-proliferative effects of retinoids are RAR-specific and insensitive to RXR-specific ligands [24]. A third major effect of retinoids on epidermis is disorganization and increased fragility of the stratum corneum, which is the outermost, nonliving layer of epidermis that acts as a water permeability barrier for skin [134]. A loss of adhesion among the non-living cells or corneocytes of the stratum corneum in the hair duct is part of the mechanism of topical retinoid therapy in acne [135]. Retinoid clearing of ductal blockage also occurs in the rhino mouse, a version of the hairless mouse which has a grotesque, wrinkled appearance due to clogging and swelling of residual hair ducts with corneocytes from the surrounding ductal epithelium. Clearance of the accumulated ductal corneocytes by topical retinoid treatment leads to a narrowing of diameter in the residual ducts and smoothening of the rhino mouse skin [136].

(i) Psoriasis

Psoriasis is a polygenic disorder with a complex pathophysiology, involving both the skin and the immune system [126,137]. The salient features of the psoriatic plaque are epidermal scaling and vastly increased thickness of the living epidermis resulting from hyperproliferation of epidermal keratinocytes. Epidermal differentiation is also markedly affected, and the granular layer of the epidermis is generally absent. Injury to the skin induces a large number of genes also highly expressed in psoriatic epidermis, suggesting close phenotypic parallels between the two types of epidermal hyperproliferation [127,138]. The psoriatic plaque is also severely inflamed due to epidermal and dermal infiltration of granulocytes, macrophages, and T cells [139]. The infiltration by T-cells (CD4$^+$ in the dermis and CD8$^+$ in the epidermis), suggests an auto-immune component in the disease, and immunosuppressive therapy with cyclosporin is indeed very effective in controlling psoriasis [140]. More recently, it has been shown that either blockage of the IL-2 receptor or inhibition of the T-cell co-stimulatory molecule CTLA-4 can reverse the symptoms of psoriasis [141]. Although the most severe forms of psoriasis involve a large percentage of the body surface area, the disease is generally localized in discrete plaques, with an obvious border between involved and uninvolved skin. These data suggest that the putative T-cell targets in psoriasis are much more highly expressed in hyperproliferative than in normal skin. Significantly, psoriasis can be induced

in susceptible individuals by injury to skin [142] (the Koebner effect), an observation which also suggests that some cases of psoriasis involve T-cell reaction to antigens expressed only in hyperproliferative epidermis and skin.

Both topical and systemic retinoids are used clinically to treat psoriasis. Etretinate was first introduced [143] followed by its free acid, acitretin, as oral therapy [144]. TTNPB 10, a highly potent RAR agonist, was very effective in clinical trials [145], but was not developed further because of associated toxicity. 13-cis RA is also used for pustular psoriasis, in which there is widespread, acute, epidermal accumulation of granulocytes [146]. Oral retinoids are rated second only to UV based therapies such as Goeckerman and PUVA for long term maintenance of remission and are superior to cyclosporin in this respect. Among topical therapies, the RAR-β,γ-selective agonist tazarotene is effective in the treatment of plaque psoriasis and provides a prolonged maintenance of therapeutic effect. Topical steroids are very effective as psoriasis therapy in the short term, but their use is limited because of skin thinning and skin atrophy following prolonged application and also because of the rapid relapse that often follows cessation of therapy [3].

A detailed study of oral etretinate action in psoriasis shows that altered keratinocyte differentiation, inhibition of cell proliferation, and depression of T-cell infiltration all take place to a significant level as a result of drug treatment [127]. Other effects include down-regulation of the inflammatory markers HLA-DR and ICAM-1, as first described for topically applied tazarotene [126]. Mechanistic studies suggest retinoids have a direct effect on cell proliferation. In particular, tazarotene has been shown to induce a growth inhibitory gene, TIG-3, both in treated lesional epidermis and in cultured human keratinocytes [147]. In addition, retinoids inhibit a number of genes turned on as a result of changes in epidermal hyperproliferation. These genes appear to be a part of the altered differentiation phenotype associated with rapidly proliferating epidermis and have been termed markers of "regenerative maturation" [22,127,138]. Many of these markers are also highly inducible during differentiation of keratinocyte cultures and are inhibited by tazarotene or ATRA, suggesting that retinoid inhibition of markers in keratinocyte culture may have predictive value for identification of retinoids effective in psoriasis [16,22,148].

HLA-DR and ICAM-1 are induced by interferon-γ and are involved in immune activation or T-cell recruitment, and their inhibition within two weeks of either topical or oral retinoid treatment suggests that the T- and inflammatory-cell migration to the skin may be rapidly down-regulated after retinoid treatment [126,127]. The infiltration of CD11b positive cells, which include granulocytes and monocytes, is suppressed by oral acitretin and by liarozole, an inhibitor of P450-mediated ATRA breakdown in vivo, that has also been used successfully in the treatment of psoriasis [149,150]. In addition, etretinate therapy is associated with decreased numbers of $CD8^+$ and $CD3^+$ T-cells in both the epidermis and dermis, demonstrating that there is a reduction in T-cell trafficking [127]. Although retinoids are not anti-inflammatory agents in the global sense of corticosteroids, several studies show that they inhibit neutrophil and macrophage influx into normal skin. A particularly remarkable finding is that migration of these cells through tape-stripped epidermis (in which the outer barrier layer or stratum corneum is removed) is >90% inhibited in skin of male subjects tested following a two-month course of 13-cis RA for acne [151]. These data suggest that changes in gene expression in epidermis or in skin-specific cell-surface adhesion proteins on the neutrophils themselves may have a major effect on accumulation of these inflammatory cells in skin. Additional studies report that both oral etretinate and topical retinoids inhibit neutrophil accumulation in epidermis in response to leukotriene B_4 [152,153]. In addition, retinoids inhibit the expression of inducible nitric oxide synthesis and other selected inflammatory mediators [154].

Based on these and other findings, several retinoids have been tested in animal models of arthritis with decidedly mixed but somewhat encouraging results. Whereas 13-cis RA is effective in adjuvant-induced rat arthritis, it significantly exacerbates collagen-induced arthritis in rat [155,156]. Other synthetic retinoids have been used successfully to ameliorate collagen-induced arthritis in rat [157]. Receptor-specific retinoids may have a future as anti-inflammatory agents if pro-arthritic or pro-inflammatory activities can be eliminated.

(ii) Acne

Acne is a disease of the hair follicle and its associated appendage, the sebaceous gland. The sebaceous gland produces sebum, a mixture of lipids that is secreted through the hair duct onto skin. Sebum production first becomes significant during puberty, primarily on the face and upper chest and back. As androgen levels rise during the early stages of puberty, sebaceous gland size and sebum production increase [5,158]. Mild, non-inflammatory acne develops as the hair duct becomes plugged due to overproduction and compaction of the epithelial cells that line the hair shaft, trapping the sebum. Sebum is also a nutrient source for the resident skin bacterium, *Propionobacterium acnes*, which may begin to populate the duct [135]. Rupture of the duct or the presence of secreted products of *P. acnes* infection are causes of more severe inflammatory acne. The most serious form of acne, nodulocystic acne, is a disfiguring, potentially scarring, and socially traumatizing condition. Its peak incidence is during late adolescence and early adulthood. It is absolutely androgen dependent and may in some cases reflect underlying endocrinological problems or use of anabolic steroids. By and large, however, normal adult androgen levels in adult males and females suffice for normal sebum production and susceptibility to the disease [158]. The sebaceous gland expresses high levels of both androgen receptor and the enzyme 5α-reductase, which converts testosterone and other androgens to high affinity ligands for the androgen receptor [159,160].

The mechanisms of action of topical and oral retinoids overlap but also demonstrate one very significant difference: only the orally administered retinoid 13-cis RA effectively blocks sebum secretion and treats noculocystic acne. Both classes of retinoids reduce accumulation of the cornified epithelial cells lining the hair duct by reducing cohesion among the corneocytes of the dead layer in an analogous manner to events in the epidermal stratum corneum following topical retinoid treatment [9], and this is effective for the milder acne vulgaris. Because turnover of the ductal epithelial cells is higher in acne than in normal follicles [161], the anti-hyperproliferative effects of topical retinoids may be helpful. The unique effects of oral 13-cis RA on the sebaceous gland itself include hypoplasia, reduced thymidine incorporation into basal cells, and a switch in the cell population from a majority of differentiated cells--producing and storing sebum in large droplets--to an increased proportion of basal undifferentiated cells [162]. These changes lead to ~85% reduction in sebum accumulation at the surface of the skin within two weeks and to significant clearing of acne within 8 weeks [1,163]. Importantly, the therapeutic effect continues and even improves after the cessation of treatment even though, during this time, normal or near normal

sebum production is restored. The data suggest that the presence of high levels of sebum contribute to acne pathogenesis but that sebum itself doses not directly cause acne. By drastically reducing sebum levels, the acne lesion is able to heal and thus normal sebum production can be tolerated. The well-established long-term benefits of 13-cis RA treatment [5] may also result from fundamental changes to the pilosebaceous structure, but the nature of these changes remain speculative. What is remarkable, however, is that other retinoids tested orally. such as etretinate, 9-cis RA and ATRA, neither reduce sebum secretion nor improve nodulocystic acne to a significant extent [164,165] even though these retinoids, at the same doses, are therapeutically effective in other diseases. These data strongly implicate inhibition of sebum secretion as an essential step in systemic retinoid therapy for nodulocystic acne [163,164].

ATRA, adapalene, and tazarotene are all used clinically as topical anti-acne agents. Their relative potencies have been compared according to concentration of topical retinoid causing an 80% inhibition of TPA-inducible ODC activity in mouse epidermis (see Table 4). Tazarotene is about 1.5 – 2 fold more potent than ATRA, which in turn is about 20 fold more potent than adapalene. For topical treatment of acne, 0.1% adapalene appeared to be superior to .025% ATRA [166,167]. The basis of the greater efficacy and tolerability of adapalene relative to ATRA has been suggested to be its relative selectivity for binding and transactivation of RAR-β and RAR-γ in comparison to RAR-α. It is not clear how this change brings an improvement in acne therapy since RAR-γ appears to be primarily responsible for skin irritation [42].

(iii) Reversal of photoaging

Kligman first suggested in 1986 that topical ATRA could be effective in reversing the effects of sun exposure-induced aging (photoaging) of skin [168]. ATRA produces a thickening of the epidermis and an overall improvement in epidermal histology as well as trophic effects on dermal fibroblasts, including new collagen deposition [6]. Extensive clinical trials have shown that outward signs of photoaging, such as fine wrinkling of the skin, are also reversed during prolonged therapy (>6 months) [169], and a topical ATRA cream (Renova or Retinova) has been approved for treatment of photoaging. Trophic effects, such as epidermal hyperplasia [131] and increased synthesis of dermal extracellular matrix proteins such as collagen and elastin [170] may bring about the gradual remodeling of the damage produced by

UV-induced photoaging. The probable nature of UV-induced injury can be appreciated from detailed studies of the initial biochemical events following UV exposure to human epidermis. Stromelysin, gelatinase, and collagenase as well as the regulatory proteins AP-1 and NF-κB are induced within 15 minutes to 24 hours [171]. These data are consistent with known effects of UV light in cultured cells and with the observation that breakdown and disorganization of extracellular matrix is characteristic of photoaged skin. Retinoids are well-characterized inhibitors of AP-1 activity, and pretreatment of UV-treated skin reduces AP-1 binding to DNA as well as the UV-induced expression of collagenase and gelatinase [171]. The concept that retinoic acid levels may be physiologically important in the control of photoaging is supported by the finding that levels of RXR-α and RAR-γ protein and mRNA (along with ATRA-mediated inhibition of gene expression) are sharply reduced after UV treatment. ATRA pretreatment of skin prevents the destruction of receptor protein by UV light [172].

c. Cancer Therapy

Characterization of receptor-specific retinoids and an appreciation of the potential of combination therapies with other nuclear receptor ligands has produced some notable advances in the area of retinoids and cancer since an earlier review [173]. Acute promyelocytic leukemia (APL) is a rare disease that is characterized by a reciprocal chromosomal translocation between the RAR-α (chromosome 17) and PML (chromosome 15) genes [174]. Expression of the mutant PML-RAR-α fusion protein acts as a dominant negative inhibitor of retinoid signaling by recruiting the negative co-activators N-CoR and SMRT along with histone de-acetylase [175], preventing the differentiation of the APL cell that might normally occur in the presence of endogenous ATRA. ATRA therapy has been successful in inducing remission in APL patients along with supportive chemotherapy [176], presumably by overcoming the negative effects of PML-RAR-α with supraphysiological concentrations of retinoid ligand. However, this therapy is somewhat limited by the toxic effects of ATRA and the development of drug resistance in some patients due to autoinduction of drug metabolism [177]. The RAR-α selective synthetic retinoid Am 80 (42) has been used successfully in inducing complete remission in the patients who have failed on ATRA therapy [178]. The apparent critical involvement of RAR-α in lymphoid and hematopoietic differentiation

suggests a beneficial role for RAR-α specific agonists in other leukemias as well [179]. The levels of RAR-α are higher in estrogen receptor (ER)-positive breast cancer cell lines than in ER-negative cell lines and tumors [180,181]. Retinoids that bind to RAR-α have a growth inhibitory effect on the ER-positive MCF-7 breast cancer cells [182]. The ER-negative SKBR-3 cells, however, express high levels of RAR-α protein and respond to RAR-α selective retinoids [183] in the same manner as the ER-positive MCF-7 breast cancer cells, suggesting that RAR-α selective retinoids offer significant advantages in ER-negative tumors which are not sensitive to conventional anticstrogen treatment.

Recent studies have also demonstrated that a combination of ATRA and ligands for PPAR-γ can induce apoptosis and suppress expression of bcl-2 in breast cancer cell lines *in vitro* and in mouse xenograft to a much greater extent than ligands for either receptor alone [184]. ATRA and certain Vitamin D3 analogues have also been found to have synergistic effects in regulation of the growth of the prostate cancer cell line, LNCaP [185]. The mechanisms and receptor selectivities of these retinoid effects remain to be explored but these findings suggest that non-cytotoxic hormone receptor therapy has the potential for wider application in cancer.

Retinoids are among the most widely studied cancer chemopreventive agents [128] and ATRA is the best-studied inhibitor of TPA-mediated tumor promotion in skin [133]. Both experimental studies and observations on the natural course of tumor development in human suggest that activation of RAR-β could be an important pathway by which retinoids inhibit cancer cell growth or progression. For example, loss of RAR-β expression is implicated in hepatocarcinogenesis [186], oral leukoplakia [187] and in head and neck tumors [188]. Isotretinoin (4) has been used successfully in the treatment of oral · leukoplakia with concomitant restoration of normal RAR-β expression [187] and also as a chemopreventive agent for second tumors in head and neck cancers [4]. Experimental studies show that loss of RAR-β expression is associated with loss of growth control [44,45], and therefore RAR-β may act as a tumor suppressor in the presence of ligand. For these reasons, targeting of RAR-β may be useful both for cancer therapy and cancer chemoprevention.

RXR-specific retinoids may also have a place in cancer chemotherapy. LGD 1069 (8) can cause complete regression of mouse mammary tumors induced by *N*-nitroso-*N*-methylurea in rats. Significantly, RAR activation by LGD 1069 appears limited as judged by absence of th usual side effects of hypervitaminosis A. In addition, tamoxifen, a partial estrogen antagonist, which also causes these tumors to regress, can act synergistically with LGD 1069 when the two drugs are combined [189]. Liposarcoma may also be RXR-responsive based on its response to troglitazone, which activates the RXR heterodimeric partner, PPAR-γ. As noted above, both RXR-specific retinoids and PPAR-γ ligands induce cultured adipocyte differentiation. In cultured liposarcomas, PPAR-γ ligands induce a similar differentiation. Recently, differentiation of high-grade (undifferentiated) liposarcoma has also been demonstrated to occur at the molecular and histological level in patients treated with troglitazone, a PPAR-γ agonist [190].

d. Emphysema and Bronchopulmonary Dysplasia of Newborns

Retinoids can enhance lung alveolus formation in animal models and in very premature infants. Massive Vitamin A supplementation has been shown to reduce the chances of chronic lung disease in extremely-low-birthweight infants to a small but medically and statistically significant extent [191]. Under circumstances where lung development is experimentally impeded in dexamethasone-treated newborn rats, ATRA is able to improve gas exchange through the lungs and increase the overall formation of alveoli [192]. A similar beneficial effect was observed in elastase-induced emphysema in the rat, in which several parameters of lung function, including total alveolar surface area, were significantly improved by subsequent daily injections of ATRA [193].

e. Vascular Effects of Retinoids

Retinoids both promote and inhibit angiogenesis depending on the system tested [194,195]. In the chorioallantoic membrane of the chick embryo, several RAR-specific retinoids, including Am 580 and Am 80, prevented ongoing vascularization when implanted in polymeric pellets [194]. On the other hand, ATRA can enhance the formation of capillary-like structures (a component step of angiogenesis) from microvascular endothelial cells cultured on matrices of crosslinked human fibrin [195]. The exact mechanisms and receptor specificities involved are not known. In addition, retinoids inhibit the growth of vascular smooth muscle cells [196,197], and studies in the rat show
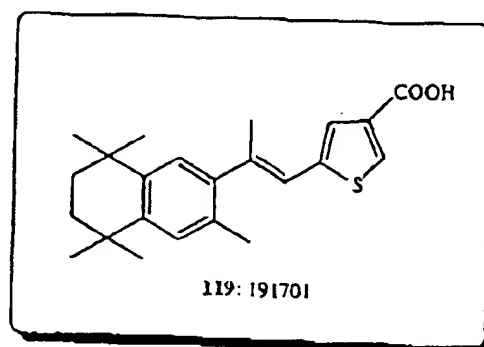
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that the vascular complications of coronary angioplasty, in which overgrowth of smooth muscle cells may occur, can be partially overcome by ATRA treatment, increasing vascular patency compared to controls [198]. These experimental systems will be useful for characterization of novel receptor and function selective retinoids.

f. Diabetes and Metabolic Disease

Kliewer and co-workers [32] first reported that thiazolidinediones (TZDs), which lower blood glucose in animal models of Type 2 diabetes, also bind the nuclear receptor PPARγ. Heterodimerization with RXR is required for PPARγ activity [199] and the hypothesis that RXR-specific compounds would have similar properties to the TZDs in Type 2 diabetes was subsequently established [7]. Type 2 or adult-onset diabetes is a progressive metabolic disease in which liver, muscle, and fat, the major tissues involved in regulation of blood glucose by insulin, become increasingly resistant to its action [200]. Blood glucose levels are elevated when the pancreatic β-cells in the islet of Langerhans become incapable of releasing the increased insulin required to compensate for peripheral insulin resistance. Serum lipids, including circulating triglycerides, are generally elevated as well. The consequences of extreme blood glucose elevation can be diabetic ketosis and death. Milder but nonetheless pathologic elevations in the range of 150 to 400 mg/dl glucose (normal fasting glucose levels are considered to be below 120 mg/dl) progressively disrupt microcirculation in kidney and retina through relatively non-specific glycation of extracellular matrix proteins. Aggressive maintenance of blood glucose levels at near normal levels by insulin or other therapies is strongly correlated to a reduction in these toxic effects [201].

LG268, the most widely investigated RXR-specific ligand, has several beneficial effects in animal models of diabetes. In addition to lowering blood glucose levels in *db/db* mice, which are obese and diabetic due to a mutation in the leptin receptor [7], LG268 also slows the destruction of pancreatic β-cells which otherwise proceeds very rapidly [8]. The basis of RXR action in diabetes is not well understood and most effects described to date have been based on studies of TZDs such as BRL 49653 and troglitazone. Like the TZDs, RXR-specific retinoids may also increase insulin sensitivity. This is consistent with improved glucose tolerance (the rate at which an exogenous bolus of glucose is removed from the circulation)

and by a lowering of serum insulin levels simultaneously with blood glucose, suggesting that the diabetic animal is more insulin responsive [7,8]. In cell culture, RXR-specific retinoids as well as TZDs induce differentiation of cultured 3T3-L1 cells, a pre-adipocyte or fibroblast cell line, into heavily fat-laden adipocytes [115]. If pre-treated with tumor necrosis factor-α, the 3T3-L1 cell becomes resistant to the acute stimulation of tyrosine phosphorylation by insulin. In this model of insulin resistance, both TZDs and LG 268 partially reverse the loss of sensitivity of insulin receptor to insulin as measured by tyrosine phosphorylation of specific downstream substrates [202]. Some potential therapeutic effects of RXR ligands may not necessarily be mediated by the RXR/PPAR-γ heterodimer. For example, intracellular accumulation of fatty acids has been proposed to contribute to pancreatic β-cell failure. Isolated pancreatic islets (containing the β-cells) respond to 9-cis RA and clofibrate, a PPAR-α ligand, by inducing both overall fatty acid oxidation and the related enzymes acyl-CoA oxidase and carnitine palmitoyl transferase I, suggesting that RXR ligands may directly contribute to protection of the β-cell [203]. The RXR ligand AGN 191701 (119) induces acyl-CoA oxidase in rat liver [26] and fatty oxidation is elevated by LG 268 but not the PPAR-γ agonists BRL 49653 or GW1929 in homogenates of liver in treated *db/db* mice [8], consistent with the findings in pancreas. RXR ligands also reverse diabetic dyslipidemia in *db/db* mice, and serum triglycerides in the diabetic animals are significantly lower than controls with ongoing treatment, although in one report it appeared that LG 268 was less effective than PPAR-γ ligands for this response [8]. Increased RXR ligand-inducible liver oxidation of free fatty acids may have a role in the reported triglyceride lowering.

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Other potential metabolic disease indications for RXR ligands include treatment of polycystic ovary syndrome by reversal of insulin resistance. In

polycystic ovary syndrome, hyperinsulinemia leads to elevated androgen production in the ovary: other forms of diabetic therapy, such as metformin or the TZD troglitazone, reverse the hyperandrogenism as the insulin resistance is reduced, and presumably an RXR-specific retinoid can act similarly [204].

5. SIDE EFFECTS OF RETINOID THERAPY

Therapeutic retinoids have a number of well documented side effects, including irritation of mucous membranes and skin (mucocutaneous toxicity), elevation of serum triglycerides (hypertriglyceridemia), headache, central hypothyroidism, and with longer-term treatment, joint pain, and modifications to bone including both ossification and osteoporosis. Both animal studies and clinical testing with RAR and RXR selective compounds have provided some information on the receptor subtypes responsible. The most rapid in onset and most universally reported of retinoid side effects is mucocutaneous toxicity, in which skin and mucous membranes (especially lips) become dry, flaky, sensitive and irritated [9]. Both topical and systemic retinoids have this effect. In the case of topical retinoids, initial speculation concerned the possibility that the amphipathic character of ATRA, like common irritants such as the detergent sodium lauryl sulfate, could be responsible for the irritation [205]. Retinoid-induced cutaneous irritation in mouse is completely blocked by the RAR-specific antagonist AGN 193109 and can be produced by a very low concentration (0.0003%) of the potent retinoid agonist TTNPB, clearly demonstrating that the effect is receptor-mediated [10]. Since retinoid topical irritation is unaffected by an RARα-specific antagonist in mouse [43], and since RARβ is not expressed in epidermis [42], it appears that RARγ is the critical target for retinoid topical irritation. These findings indicate that an RAR-α agonist would be devoid of mucocutaneous toxicity if used for a clinical indication.

Serum hypertriglyceridemia in the rat can be induced by RAR-specific retinoids [10]. In human, monitoring of serum triglycerides during early stages of retinoid treatment is vital since some patients may experience significant elevation (>400 mg/dl) [146]. TTNPB 10 appears not to elevate triglycerides in human, but dose limitation due to other toxicities may explain the apparent absence of a TTNPB effect [10,145]. Other changes in lipoprotein profiles have been reported with 13-cis RA, including increases in serum cholesterol and LDL [206,207], but hypertriglyceridemia is

considered the most serious since acute pancreatitis, a potentially lethal condition, may occur as a result. Moderate elevation of triglycerides has been treated symptomatically with fibrates, which increase the oxidative breakdown of fatty acids in the liver [208]. Interestingly, the RAR-RXR pan-agonist 9-cis RA induces substantially higher triglyceride levels in rat than RAR agonists, suggesting that ligand activation of RXR may potentiate the RAR effect [10]. Under the conditions tested (3 consecutive days of treatment) RXR ligands, including LG 268, alone did not appear to cause hypertriglyceridemia [10]. Clinical studies with 9-cis RA have shown that hypertriglyceridemia can be substantially elevated in a small number of patients [209]. Surprisingly, a recent report finds that lipoprotein (a), which is strongly associated with risk of cardiovascular disease, is markedly reduced in acne patients following 30 days of 13-cis RA therapy [207], suggesting that the balance of changes in lipid profile for most patients will not necessarily increase the risk of cardiovascular disease.

Several mechanisms have been proposed to account for retinoid-induced serum triglyceride elevation. An obvious retinoid target is lipoprotein lipase (LPL), which is bound on the surface of endothelial cells, primarily in muscle and fat, and releases fatty acids from the lipoprotein-bound triglycerides for uptake into the surrounding tissue. LPL inhibition therefore has the effect of slowing the removal of lipoprotein-bound triglycerides from serum. An early study reported no change in total endothelial surface LPL (following intravenous injection of heparin which releases LPL from its cell surface anchor) in human patients treated with 13-cis RA [206]. A second human study reported that clearance of injected triglycerides was reduced by 13-cis RA treatment as would be expected, and that total LPL activity from muscle biopsies was also decreased [210]. 13-cis RA treatment of rats also inhibits LPL activity [211]. Serum triglycerides may also be elevated as a result of changes in the protein composition of the chylomicrons and the circulating lipoprotein, very low density lipoprotein (VLDL), which are the primary carriers of triglycerides in serum. In humans, 13-cis RA induces expression of apo-CIII, which is incorporated into chylomicrons and VLDL and can prolong the half-life of the two lipoproteins in the circulation [212]. ATRA induces expression apo-CIII mRNA in the intestine of the Vitamin A-deficient rat, presumably increasing apo-CIII in chylomicrons [213]. Other work suggests that induction of apo-CIII is primarily RXR-mediated in liver cell cultures [212]. These data

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may help to explain why the RAR and RXR active retinoid 9-cis RA has a more substantial effect on triglyceride elevation than RAR-selective retinoids alone.

The effects of retinoids on embryonic bone development have been studied in depth [214]. Retinoids accelerate fetal chondrocyte differentiation both in culture and during development. This action most likely underlies the extensive skeletal and craniofacial defects associated with retinoid embryotoxicity in humans. Premature closure of the bone end plate (endochondreal ossification) can stunt the growth of children taking retinoids chronically for inherited disorders of epidermal differentiation and has been shown to be RAR-dependent in guinea pigs [215]. In adult, both the formation of bony spurs and osteoporosis are reported: generally these effects are only observed in patients treated at high doses for more than one year [216].

A previously unappreciated effect of RXR ligands is central hypothyroidism. Very high doses of LG 1069 were recently shown to lower serum T_3 and T_4 by about 50%, producing mild symptoms of hypothyroidism [12]. At the same time, TSH (thyroid-stimulating hormone) levels are depressed as much as 40 fold, indicating that RXR ligands have a central effect on the pituitary gland rather than a peripheral effect on the thyroid. Inhibition of TSH production by T_3 is the physiological feedback loop that controls thyroid function, and, based on studies of isolated pituitary cells, appears to require expression of RXRγ within the TSH-producing cells of the pituitary (the pituitary thyrotropes) [46]. Since hypothyroidism is only observed with very high doses of LG 1069 [12], it is possible that RXR-induced hypothyroidism could be relatively uncommon in general medical usage of these compounds.

6. CONCLUSION

Retinoid medicinal chemistry has become a vastly more powerful and diverse area of research since the synthesis of receptor selective agonists and antagonists began in earnest. Although more work will be required to find both agonists and antagonists for RAR-β, RAR-γ, and the RXR subtypes, it is clear from work with RXR agonists and RAR inverse agonists that entirely new therapeutic areas and approaches are becoming accessible which could not have been imagined as recently as five years ago.

LIST OF ABBREVIATIONS

ATRA = All trans retinoic acid

TR = Thyroid receptor

TZD = Thiazolidinedione

AF-1 = Activation function −1

AF-2 = Activation function −2

HAT = Histone Acetyl transferase

HMG-1 = High mobility group protein −1

TTNPB = [(E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthelenyl)-propen-1yl] benzoic acid

TPA = 12-O-tetradecanoyl phorbol-13-acetate

ODC = Ornithine decarboxylase

SAR = Structure-activity relationship

MRP-8 = Migratory inhibitory factor-related protein 8

NFIL-6 = Nuclear factor-interleukin-6

HB-EGF = Heparin-binding epidermal growth factor

ER = Estrogen receptor

LPL = Lipoprotein lipase

7. REFERENCES

[1]   Shalita, A. R.; Armstrong, R. B.; Leyden, J. J.; Pochi, P. E.; Strauss, J. S. *Cutis*, 1988, 42, 1.

[2]   Warrell, R. P., Jr. *Annu. Rev. Med.*, 1996, 47, 555.

[3]   Koo, J.; Lebwohl, M. *J. Am. Acad. Dermatol.*, 1999, 41, 51.

[4]   Hong, W. K.; Lippman, S. M.; Itri, L. M.; Karp, D. D.; Lee, J. S.; Byers, R. M.; Schantz, S. P.; Kramer, A. M.; Lotan, R.; Peters, L. J.; et al. *N. Engl. J.Med.*, 1990, 323, 795.

[5]   Leyden, J. J. *N. Engl. J. Med.*, 1997, 336, 1156.

[6]   Griffiths, C. E. M.; Russman, A. N.; Majmudar, G.; Singer, R. S.; Hamilton, T. A.; Voorhees, J. J. *N. Engl. J. Med.*, 1993, 329, 530.

[7]   Mukherjee, R.; Davies, P. J. A.; Crombie, D. L.; Bischoff, E. D.; Cesario, R. M.; Jow, L.; Hamann,

L. G.; Boehm. M. F.; Mondon, C. E.; Nadzan, A. M.; Paterniti, J. R.. Jr. Heyman. R. A. *Nature*. 1997. *386*, 407.

[8]   Lenhard. J. M.; Lancaster. M. E.; Paulik. M. A.; Weiel. J. E.; Binz, J. G.; Sundseth, S. S.; Gaskill. B. A.; Lightfoot, R. M.; Brown, H. R. *Diabetologia*. 1999. *42*, 545.

[9]   Elias, P. M. *Dermatologica*. 1987. *175 suppl. 1*, 28.

[10] Standeven. A. M.; Beard, R. L.; Johnson, A. T.; Boehm. M. F.; Escobar. M.; Heyman, R. A.; Chandraratna. R. A. S. *Fundam. Appl. Toxicol*.. 1996. *33*. 264.

[11] Armstrong, R. B.; Ashenfelter, K. O.; Eckhoff. C.; Levin, A. A.; Shapiro, S. S.; in The Retinoids. Biology. Chemistry, and Medicine; 2nd ed. ed.; Sporn, M. B.. Roberts. A. B. and Goodman, D. S.. Ed.; Raven Press: New York. 1994. pp. 545.

[12] Sherman, S. I.; Gopal, J.; Haugen, B. R.; Chiu, A. C.; Whaley, K.; Nowlakha, P.; Duvic, M. *N. Engl. J. Med.*, 1999, *340*, 1075.

[13] Mitchell, A. A.; VanBennekom. C. M.; Louik, C. *N. Engl. J. Med.*, 1995, *333*, 101.

[14] Johnson, A. T.; Wang, L.; Standeven, A. M.; Escobar, M.; Chandraratna, R. A. S. *Bioorg. Med. Chem.*, 1999, *7*, 1321.

[15] Klein, E. S.; Pino, M. E.; Johnson, A. T.; Davies. P. J. A.; Nagpal, S.; Thacher, S. M.; Krasinski, G.; Chandraratna, R. A. S. *J. Biol. Chem.*, 1996, *271*, 22692.

[16] Thacher, S. M.; Nagpal, S.; Klein, E. S.; Arefieg, T.; Krasinski, G.; DiSepio, D.; Agarwal, C.; Johnson, A. T.; Eckert, R. L.; Chandraratna, R. A. S. *Cell Growth & Differentiation*, 1999, *10*, 255.

[17] Mangelsdorf, D. J.; Umesono, K.; Evans, R. M.; in The Retinoids: Biology, Chemistry and Medicine; 2nd ed.; Sporn, M. B., Roberts, A. B. and Goodman, D. S., Ed.; Raven Press: New York. 1994. pp. 319.

[18] Allenby, G.; Bocquel, M. T.; Saunders, M.; Kazner, S.; Speck, J.; Rosenberger, M.; Lovey. A.; Kastner, P.; Grippo, J. F.; Chambon, P.; Levin, A. A. *Proc. Natl. Acad. Sci.*, 1993, *90*, 30.

[19] Allegretto, E. A.; McClurg, M. R.; Lazarchik, S. B.; Clemm, D. L.; Kerner, S. A.; Elgort, M. G.; Boehm, M. F.; White, S. K.; Pike, J. W.; Heyman, R. A. *J. Biol. Chem.*, 1993, *235*, 26625.

[20] Dilworth, F. J.; Fromental-Ramain, C.; Remboutsika, E.; Benecke, A.; Chambon, P. *Proc. Natl. Acad. Sci. U S A*, 1999, *96*, 1995.

[21] Gendimenico, G. J.; Stim, T. B.; Corbo, M.; Janssen, B.; Mezick, J. A. *J. Invest. Dermatol*., 1994, *102*, 676.

[22] Nagpal, S.; Thacher, S. M.; Patel, S.; Friant, S.; Malhotra, M.; Shafer, J.; Krasinski, G.; Asano, A. T.; Teng, M.; Duvic, M.; Chandraratna, R. A. S. *Cell Growth & Differentiation*. 1996. *7*, 1783.

[23] Kurokawa, R.; DiRenzo, J.; Boehm, M. F.; Sugarman, J.; Gloss, B.; Heyman, R. A.; Rosenfeld, M. G.; Glass, C. K. *Nature*. 1994. *371*, 528.

[24] Thacher, S. M.; Standeven, A. S.; Athanikar, J.; Kopper, S.; Castilleja, O.; Escobar, M.; Beard, R. L.; Chandraratna, R. A. S. *J. Pharm. Exp. Ther.*, 1997, *282*, 528.

[25] Westin, S.; Kurokawa, R.; Nolte, R. T.; Wisely, G. B.; McInerney, E. M.; Rose, D. W.; Milburn, M. V.; Rosenfeld, M. G.; Glass, C. K. *Nature*, 1998, *395*, 199.

[26] Standeven, A. M.; Escobar, M.; Beard, R. L.; Yuan, Y.-D.; Chandraratna, R. A. S. *Biochem. Pharmacol*.. 1997, *54*, 517.

[27] Mukherjee, R.; Strasser, J.; Jow, L.; Hoener, P.; Paterniti, J. R., Jr.; Heyman, R. A. *Arterioscler. Thromb. Vasc. Biol.*, 1998, *18*, 272.

[28] Kliewer, S. A.; Umesono, K.; Noonan, D. J.; Heyman, R. A.; Evans, R. M. *Nature*, 1992, *358*, 771.

[29] Mangelsdorf, D. J.; Evans, R. M. *Cell*, 1995, *83*, 841.

[30] Willy, P. J.; Mangelsdorf, D. J. *Genes Dev.*, 1997, *11*, 289.

[31] Parks, D. J.; Blanchard, S. G.; Bledsoe, R. K.; Chandra, G.; Consler, T. G.; Kliewer, S. A.; Stimmel, J. B.; Willson, T. M.; Zavacki, A. M.; Moore, D. D.; Lehmann, J. M. *Science*, 1999, *284*, 1365.

[32] Lehmann, J. M.; Moore, L. B.; Smith-Oliver, T. A.; Wilkison, W. O.; Willson, T. M.; Kliewer, S. A. *J. Biol. Chem.*, 1995, *270*, 12953.

[33] Kersten, S.; Seydoux, J.; Peters, J. M.; Gonzalez, F. J.; Desvergne, B.; Wahli, W. *J. Clin. Invest.*, 1999, *103*, 1489.

[34] Xiao, J.-H.; Durand, B.; Chambon, P.; Voorhees, J. J. *J. Biol. Chem.*, 1995, *270*, 3001.

[35] Blumberg, B.; Evans, R. M. *Genes Dev.*, 1998, *12*, 3149.

[36] Lohnes, D.; Mark, M.; Mendelsohn, C.; Dolle, P.; Dierich, A.; Gorry, P.; Gansmuller, A.; Chambon, P. *Development*, 1994, *120*, 2723.

[37] Chambon, P. *FASEB J.*, 1996, *10*, 940.

[38] Chiang, M. Y.; Misner, D.; Kempermann, G.; Schikorski, T.; Giguere, V.; Sucov, H. M.; Gage, F. H.; Stevens, C. F.; Evans, R. M. *Neuron*, 1998, *21*, 1353.

[39] Nagpal, S.; Saunders, M.; Kastner, P.; Durand, B.; Nakshatri, H.; Chambon, P. *Cell*, 1992, *70*, 1007.

[40] Dolle, P.; Ruberte, E.; Leroy, P.; Morriss-Kay, G.; Chambon, P. *Development*, 1990, *110*, 1133.

Chandraratna et al.

[41] Fisher, G. J.; Talwar, H. S.; Xiao, J. H.; Datta, S. C.; Reddy, A. P.; Gaub, M.-P.; Rochette-Egly, C.; Chambon, P.; Voorhees, J. J. *J. Biol. Chem.*, 1994, 269, 20629.

[42] Chen, J. Y.; Ostrowski, J.; Whiting, G.; Roalsvig, T.; Hammer, L.; Currier, S. J.; Honeyman, J.; Kwasniewski, B.; Yu. K.-L.; Sterzycki, R.; Kim, C. U.; Starrett, J. E.; Mansuri, M. M.; Reczek, P. J. *J. Invest. Dermatol.*, 1995, 104, 779.

[43] Standeven, A. M.; Teng, M.; Chandraratna, R. A. S. *Toxicol. Lett.*, 1997, 92, 231.

[44] Berard, J.; Laboune, F.; Mukuna, M.; Masse, S.; Kothary, R.; Bradley, W. E. C. *FASEB J.*, 1996, 10, 1091.

[45] Faria, T. N.; Mendelsohn, C.; Chambon, P.; Gudas, L. J. *J. Biol. Chem.*, 1999, 274, 26783.

[46] Haugen, B. R.; Brown, N. S.; Wood, W. M.; Gordon, D. F.; Ridgway, E. C. *Mol. Endocrinol.*, 1997, 11, 481.

[47] Kastner, P.; Mark, M.; Chambon, P. *Cell*, 1995, 83, 859.

[48] Krezel, W.; Ghyselinck, N.; Samad, T. A.; Dupe, V.; Kastner, P.; Borrelli, E.; Chambon, P. *Science*, 1998, 279, 863.

[49] Nagpal, S.; Friant, S.; Nakshatri, H.; Chambon, P. *EMBO J.*, 1993, 12, 2349.

[50] Renaud, J.-P.; Rochel, N.; Ruff, M.; Vivat, V.; Chambon, P.; Gronemeyer, H.; Moras, D. *Nature*, 1995, 378, 681.

[51] Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. *Nature*, 1998, 395, 137.

[52] McInerney, E. M.; Rose, D. W.; Flynn, S. E.; Westin, S.; Mullen, T.-M.; Krones, A.; Inostroza, J.; Torchia, J.; Nolte, R. T.; Assa-Munt, N.; Milburn, M. V.; Glass, C. K.; Rosenfeld, M. G. *Genes Dev.*, 1998, 12, 3357.

[53] Nagpal, S.; Ghosn, C.; DiSepio, D.; Molina, Y.; Sutter, M.; Klein, E. S.; Chandraratna, R. A. *J. Biol. Chem.*, 1999, 274, 22563.

[54] Heinzel, T.; Lavinsky, R. M.; Mullen, T.-M.; Soderstrom, M.; Laherty, C. D.; Torchia, J.; Yang, W.-M.; Brard, G.; Ngo, S. D.; Davie, J. R.; Seto, E.; Eisenman, R. N.; Rose, D. W.; Glass, C. K.; Rosenfeld, M. G. *Nature*, 1997, 387, 43.

[55] Horlein, A. J.; Naar, A. M.; Heinzel, T.; Torchia, J.; Gloss, B.; Kurokawa, R.; Ryan, A.; Kamei, Y.; Soderstrom, M.; Glass, C. K.; Rosenfeld, M. *Nature*, 1995, 377, 397.

[56] Nagy, L.; Kao, H. Y.; Chakravarti, D.; Lin, R. J.; Hassig, C. A.; Ayer, D. E.; Schreiber, S. L.; Evans, R. M. *Cell*, 1997, 89, 373.

[57] Hong, S.; Privalsky, M. *J. Biol. Chem.*, 1999, 274, 2885.

[58] Heyman, R. A.; Mangelsdorf, D. J.; Dyck, J. A.; Stein, R. B.; Eichele, G.; Evans, R. M.; Thaller, C. *Cell*, 1992, 68, 397.

[59] Levin, A. A.; Sturzenbecker, L. J.; Kazmer, S.; Bosakowski, T. H., C.; Allenby, G.; Speck, J.; Kratzeisen, C. I.; Rosenberger, M.; Lovey, A.; Grippo, J. F. *Nature*, 1992, 355, 359.

[60] Lehmann, J. M.; Jong, L.; Fanjul, A.; Cameron, J. F.; Lu, X. P.; Haefner, P.; Dawson, M. I.; Pfahl, M. *Science*, 1992, 258, 1944.

[61] Loeliger, P.; Bollag, W.; Mayer, H. *Eur. J. Med. Chem. Chim. Ther.*, 1980, 15, 9.

[62] Graupner, G.; Malle, G.; Maignan, G.; Lang, G.; Prunieras, M.; Pfahl, M. *Biochem. Biophys. Res. Commun.*, 1991, 179, 1554.

[63] Lehmann, J. M.; Dawson, M. I.; Hobbs, P. D.; Husmann, M.; Pfahl, M. *Cancer Res.*, 1991, 51, 4804.

[64] Delescluse, C.; Cavey, M. T.; Bernard, B. A.; Reichert, U.; Maignan, J.; Darmon, M.; Shroot, B. *Mol. Pharmacol.*, 1991, 40, 556.

[65] Charpentier, B.; Bernardon, J.-M.; Eustache, J.; Millois, C.; Martin, B.; Michel, S.; Shroot, B. *J. Med. Chem.*, 1995, 38, 4993.

[66] Chandraratna, R. A. S.; Gillett, S. J.; Song, T. K.; Attard, J.; Vuligonda, V.; Garst, M. E.; Arefieg, T.; Gil, D. W.; Wheeler, L. *Bioorg. Med. Chem. Lett.*, 1995, 5, 523.

[67] Chandraratna, R. A. S.; Henry, E.; Attard, J.; Gillett, S. J.; Song, T. K.; Garst, M. E.; Nagpal, S.; Athanikar, J.; Arefieg, T.; Gil, D. W.; Wheeler, L.; Lew-Kaya, D.; Sefton, J. *Eur. J. Med. Chem.*, 1995, 30, 506s.

[68] Nagpal, S.; Athanikar, J.; Chandraratna, R. A. S. *J. Biol. Chem.*, 1995, 270, 923.

[69] Chandraratna, R. A. S. *Br. J. Dermatol.*, 1996, 135, 18.

[70] Pegg, A. E.; McCann, P. P. *Am. J. Physiol.*, 1982, 243, C212.

[71] Matsumoto, R. M.; Sun, H.; Duff, S. B. *Pharm. Res.*, 1992, 9(Suppl). 274.

[72] Fritsch, P. O. *J. Am. Acad. Dermatol.*, 1992, 27, S8.

[73] Matsumoto, R. M.; Sun, H.; Tang-Liu, D. *Pharm. Res*, 1992, 9(Suppl), 289.

[74] Kagechika, II.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. *J. Med. Chem.*, 1988, 31, 2182.

[75] Crettaz, M.; Baron, A.; Siegenthaler, G.; Hunziker, W. *Biochem. J.*, 1990, 272, 391.

[76] Ostrowski, J.; Roalsvig, T.; Hammer, L.; Marinier, A.; Starrett, J. E., Jr.; Yu, K. L.; Reczek, P. R. *J. Biol. Chem.* **1998**, *273*, 3490.

[77] Teng, M.; Duong, T. D.; Klein, E. S.; Pino, M. E.; Chandraratna, R. A. S. *J. Med. Chem.*, **1996**, *39*, 3035.

[78] Cheng, Y.-C.; Prussof, W. H. *Biochem. Pharmacol.*, **1973**, *22*, 3099.

[79] Chen, J.-Y.; Penco, S.; Ostrowski, J.; Balaguer, P.; Pons, M.; Starrett Jr., J. E.; Reczek, P.; Chambon, P.; Gronemeyer, H. *EMBO J.*, **1995**, *14*, 1187.

[80] Johnson, A. T.; Klein, E. S.; Wang, L.; Pino, M. E.; Chandraratna, R. A. S. *J. Med. Chem.*, **1996**, *39*, 5027.

[81] Bernard, B. A.; Bernardon, J.-M.; Delescluse, C.; Martin, B.; Lenoir, M.-C.; Maignan, J.; Charpentier, B.; Pilgrim, W. R.; Reichert, U.; Shroot, B. *Biochem. Biophys. Res. Commun.*, **1992**, *186*, 977.

[82] Dawson, M. I.; -R., C.-W.; Pine, P.; Jong, L.; Hobbs, P. D.; Rudd, C. K.; Quick, T. C.; Niles, R. M.; Zhang, X.; Lombardo, A.; Ely, K. R.; Shroot, B.; Fontana, J. A. *Cancer Res.*, **1995**, *55*, 4446.

[83] Yu, K.-L.; Spinazze, P.; Ostrowski, J.; Currier, S. J.; Pack, E. J.; Hammer, L.; Roalsvig, T.; Honeyman, J. A.; Tortolani, D. R.; Reczek, P. R.; Mansuri, M. M.; Starrett, J., J. E. *J. Med. Chem.*, **1996**, *39*, 2411.

[84] Chao, W.; Hobbs, P. D.; Jong, L.; Zhang, X.; Zheng, W.; Wu, Q.; Shroot, B.; Dawson, M. I. *Cancer Lett.*, **1997**, *115*, 1.

[85] Reczek, P. R.; Ostrowski, J.; Yu, K.-L.; Chen, S.; Hammer, L.; Roalsvig, T.; Starrett Jr., J. E.; Driscoll, J. P.; Whiting, G.; Spinazze, P. G.; Tramposch, K.; Mansuri, M. M. *Skin Pharmacol.*, **1995**, *8*, 292.

[86] Swann, T. R.; Smith, D.; Tramposch, K. M.; Zusi, F. C.; ; European Patent Application, **1996**; No. 0 747 347 A1

[87] Kaneko, S.; Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *Med. Chem. Res.*, **1991**, *1*, 220.

[88] Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Shudo, K. *J. Med. Chem.*, **1988**, *31*, 2182.

[89] Yoshimura, H.; Nagai, M.; Hibi, S.; Kikuchi, K.; Abe, S.; Hida, T.; Higashi, S.; Hishinuma, I.; Yamanaka, T. *J. Med. Chem.*, **1995**, *38*, 3163.

[90] Lee, M.-O.; Hobbs, P. D.; Zhang, X.-k.; Dawson, M.; Pfahl, M. *Proc. Natl. Acad. Sci. USA*, **1994**, *91*, 5632.

[91] Lee, M.-O.; Dawson, M. I.; Picard, N.; Hobbs, P. D.; Pfahl, M. *J. Biol. Chem.*, **1996**, *271*, 11897.

[92] Johnson, A. T.; Klein, E. S.; Gillett, S. J.; Wang, L.; Song, T. K.; Pino, M. E.; Chandraratna, R. A. S. *J. Med. Chem.*, **1995**, *38*, 4764.

[93] Apfel, C.; Bauer, F.; Crettaz, M.; F mi, L.; Kamber, M.; Kaufmann, F.; LeMotte, P.; Pirson, W.; Klaus, M. *Proc. Natl. Acad. Sci. USA.* **1992**, *89*, 7129.

[94] Klaus, M.; Mohr, P.; ; European Patent Application. Nov. 10, 1993; No. EP 568898 A1

[95] Teng, M.; Duong, T. D.; Johnson, A. T.; Klein, E. S.; Wang, L.; Khalifa, B.; Chandraratna, R. A. S. *J. Med. Chem.*, **1997**, *40*, 2445.

[96] Costa, T.; Ogino, Y.; Munson, P. J.; Onaran, H. O.; Rodbard, D. *Mol. Pharmacol.*, **1992**, *41*, 549.

[97] Chidiac, P.; Hebert, T. E.; Valiquette, M.; Dennis, M.; Bouvier, M. *Mol. Pharmacol.*, **1994**, *45*, 490.

[98] Bond, R. A.; Leff, P.; Johnson, T. D.; Milano, C. A.; Rockman, H. A.; McMinn, T. R.; Apparsundaram, S.; Hyek, M. F.; Kenakin, T. P.; Allen, L. F.; Lefkowitz, R. J. *Nature.* **1995**, *374*, 272.

[99] Standeven, A. M.; Johnson, A. T.; Escobar, M.; Chandraratna, R. A. S. *Toxicol. Appl. Pharmacol.*, **1996**, *138*, 169.

[100] Agarwal, C.; Chandraratna, R. A. S.; Johnson, A. T.; Rorke, E. A.; Eckert, R. L. *J. Biol. Chem.*, **1996**, *271*, 12209.

[101] Pfahl, M. *Endocr. Rev.*, **1993**, *14*, 651.

[102] DiSepio, D.; Malhotra, M.; Chandraratna, R. A. S.; Nagpal, S. *J. Biol. Chem.*, **1997**, *272*, 25555.

[103] Schule, R.; Rangarajan, P.; Yang, N.; Kleiwer, S.; Ransone, L. J.; Bolado, J.; Verma, I. M.; Evans, R. M. *Proc. Natl. Acad. Sci.*, **1991**, *88*, 6092.

[104] Nicholson, R. C.; Mader, S.; Nagpal, S.; Leid, M.; Rochette-Egly, C.; Chambon, P. *EMBO J.*, **1990**, 4443.

[105] Yang-Yen, H. F.; Zhang, X. K.; Graupner, G.; Tzukerman, M.; Sakamoto, B.; Karin, M.; Pfahl, M. *New Biol.*, **1991**, *3*, 1206.

[106] Fanjul, A.; Dawson, M. I.; Hobbs, P. D.; Jong, L.; Cameron, J. F.; Harlev, E.; Graupner, G.; Lu, X.-P.; Pfahl, M. *Nature.* **1994**, *372*, 107.

[107] Boehm, M. F.; McClurg, M. R.; Pathirana, C.; Mangelsdorf, D.; White, S. K.; Hebert, J.; Winn, D.; Goldman, M. E.; Heyman, R. A. *J. Med. Chem.*, **1994**, *37*, 408.

[108] Boehm, M. F.; Zhang, L.; Zhi, L.; McClurg, M. R.; Berger, E.; Wagoner, M.; Mais, D. E.; Suto, C.-M.; Davies, P. J. A.; Heyman, R. A.; Nadzan, A. M. *J. Med. Chem.*, **1995**, *38*, 3146.

[109] Boehm, M. F.; Zhang, L.; Badea, B. A.; White, S. K.; Mais, D. E.; Berger, E.; Suto, C. M.; Goldman, M. E.; Heyman, R. A. *J. Med. Chem*, **1994**, *37*, 2930.

[110] Beard, R. L.; Colon, D. F.; Song, T. K.; Davies, P. J. A.; Kochhar, D. M.; Chandraratna, R. A. S. *J. Med. Chem.*, **1996**, *39*, 3556.

[111] Vuligonda, V.; Chandraratna, R. A. S.; Allergan Inc., Irvine, Calif.; United States patent, June 29, 1999; No. 5,917,082

[112] Hibi, S.; Kikuchi, K.; Yoshimura, H.; Nagai, M.; Tai, K.; Hida, T. *J. Med. Chem.*, 1998, *41*, 3245.

[113] Beard, R. L.; Gil, D. W.; Marler, D. K.; Henry, E.; Colon, D. F.; Gillett, S. J.; Arefieg, T.; Breen, T. S.; Krauss, H.; Davies, P. J. A.; Chandraratna, R. A. S. *Bioorg. Med. Chem. Lett.*, 1994, *4*, 1447.

[114] Beard, R. L.; Chandraratna, R. A. S.; Colon, D. F.; Gillett, S. J.; Henry, E.; Marler, D. K.; Song, T.; Denys, L.; Garst, M. E.; Arefieg, T.; Klein, E.; Gil, D. W.; Wheeler, L.; Kochhar, D. M.; Davies, P. J. A. *J. Med. Chem.*, 1995, *38*, 2820.

[115] Canan-Koch, S. C.; Dardashti, L. J.; Cesario, R. M.; Croston, G. E.; Boehm, M. F.; Heyman, R. A.; Nadzan, A. M. *J. Med. Chem.*, 1999, *42*, 742.

[116] Vuligonda, V.; Lin, Y.; Chandraratna, R. A. S. *Bioorg. Med. Chem. Lett.*, 1996, 213.

[117] Farmer, L. J.; Jeong, S.; Kallel, E. A.; Canan Koch, S. C.; Croston, G. E.; Flatten, K. S.; Heyman, R. A.; Nadzan, A. M. *Bioorg. Med. Chem. Lett.*, 1997, 7, 2393.

[118] Vuligonda, V.; Garst, M. E.; Chandraratna, R. A. S. *Bioorg. Med. Chem. Lett.*, 1999, *9*, 589.

[119] Canan Koch, S. S.; Dardashti, L. J.; Hebert, J. J.; White, S. K.; Croston, G. E.; Flatten, K. S.; Heyman, R. A.; Nadzan, A. M. *J. Med. Chem.*, 1996, *39*, 3229.

[120] Underwood, B. A.; Arthur, P. *FASEB J.*, 1996, *10*, 1040.

[121] Pasatiempo, A. M.; Bowman, T. A.; Taylor, C. E.; Ross, A. C. *Am. J. Clin. Nutr.*, 1989, *49*, 501.

[122] Sommer, A. *Prog. Retin. Eye Res.*, 1998, *17*, 9.

[123] Wolf, G.; *in* Nutrition and the Adult: Micronutrients; Alfin-Slater, R. B. and Kritchevsky, D., Ed.; Plenum; New York, 1980, pp. 97.

[124] Ross, A. C.; Stephensen, C. B. *FASEB J.*, 1996, *10*, 979.

[125] Kligman, A. M. *J. Invest. Dermatol.*, 1974, *62*, 268.

[126] Esgleyes-Ribot, T.; Chandraratna R. A.; Lew-Kaya, D. A.; Sefton, J.; Duvic, M. *J. Am. Acad. Dermatol.*, 1994, *30*, 581.

[127] Gottleib, S. L.; Hayes, E.; Gilleaudeau, P.; Cardinale, I.; Gottleib, A. B.; Krueger, J. G. *J. Cutan. Pathol.*, 1996, *23*, 404.

[128] Lippman, S. M.; Benner, S. E.; Hong, W. K. *J. Clin. Oncol.*, 1994, *12*, 851.

[129] Gollnick, H. P.; Dummler, U. *Clin. Dermatol.*, 1997, *15*, 799.

[130] Connor, M. J.; Ashton, R. E.; Lowe, N. J. *J. Pharm. Exp. Therap.*, 1986, *237*, 31.

[131] Xiao, J.-H.; Feng, X.; Di, W.; Peng, Z.-H.; Li, L. A.; Chambon, P.; Voorhees, J. J. *EMBO J.*, 1999, *18*, 1539.

[132] Gendimenico, G. J.; Nair, X.; Bouquin, P. L.; Tramposch, K. M. *J. Invest. Dermatol.*, 1989, *93*, 363.

[133] Verma, A. K.; Shapas, B. G.; Rice, H. M. *Cancer Res.*, 1979, *39*, 419.

[134] Elias, P. M.; Fritsch, P. O.; Lampe, M.; Williams, M. L.; Brown, B. E.; Nemanic, M.; Grayson, S. *Lab. Invest.*, 1981, *44*, 531.

[135] Jansen, T.; Plewig, G.; Kligman, A. M. *Dermatologic Therapy*, 1998, 6, 7.

[136] Mezick, J. A.; Bhatia, M. C.; Capetola, R. J. *J. Invest. Dermatol.*, 1984, *83*, 110.

[137] Elder, J. T.; Nair, R. P.; Guo, S.-W.; Henseler, T.; Christophers, E.; Voorhees, J. J. *Arch. Dermatol.*, 1994, *130*, 216.

[138] Mansbridge, J. N.; Knapp, A. M. *J. Invest. Dermatol.*, 1987, *89*, 253.

[139] Baker, B. S.; Fry, L. *Br. J. Dermatol.*, 1992, *126*, 1.

[140] Wong, R. L.; Winslow, C. M.; Cooper, K. D. *Immunology Today*, 1993, *14*, 69.

[141] Abrams, J. R.; Lebwohl, M. G.; Guzzo, C. A.; Jegasothy, B. V.; Goldfarb, M. T.; Goffe, B. S.; Menter, A.; Lowe, N. J.; Krueger, G.; Brown, M. J.; Weiner, R. S.; Birkhofer, M. J.; Warner, G. L.; Berry, K. K.; Linsley, P. S.; Krueger, J. G.; Ochs, H. D.; Kelley, S. L.; Kang, S. *J. Clin. Invest.*, 1999, *103*, 1243.

[142] Krueger, G. G.; *in* Immune Mechanisms in Cutaneous Disease; Norris, D. A., Ed.; Marcel Dekker: New York, 1989, pp. 425.

[143] Ellis, C. N.; Voorhees, J. J. *J. Am. Acad. Dermatol.*, 1987, *16*, 267.

[144] Larsen, F. G.; Vahlquist, C.; Andersson, E.; Torma, H.; Kragballe, K.; Vahlquist, A. *Acta. Derm. Venereol.*, 1992, *72*, 84.

[145] Merot, Y.; Camenzind, M.; Geiger, J.-M.; Saurat, J.-H. *Acta Derm. Venereol.*, 1987, *67*, 237.

[146] Gollnick, H. P. *Br. J. Dermatol.*, 1996, *135 Suppl 49*, 6.

[147] DiSepio, D.; Ghosn, C.; Eckert, R.; Deucher, A.; Robinson, N.; Duvic, M.; Chandraratna, R. A. S.; Nagpal, S. *Proc. Natl. Acad. Sci. USA*, 1998, *95*, 14811.

[148] Hohl, D.; de Viragh, P. A.; Amiguet-Barras, F.; Gibbs, S.; Backendorf, C.; Huber, M. *J. Invest. Dermatol.*, 1995, *104*, 902.

[149] Kuijpers, A. L.; Van Pelt, J. P.; Bergers, M.; Boegheim, P. J.; Den Bakker, J. E.; Siegenthaler, G.; Van de Kerkhof, P. C.; Schalkwijk, J. *Br. J. Dermatol.*, 1998, *139*, 380.

[150] van Pelt, J. P.; de Jong, E. M.; de Bakker, E. S.; van de Kerkhof, P. C. *Skin. Pharmacol. Appl. Skin Physiol.*, 1998, *11*, 70.

[151] Norris, D. A.; Osborn, R.; Robinson, W.; Tonnensen, M. G. *J. Invest. Dermatol.*, 1987, *89*, 38.

[152] Lammers, A. M.; van de Kerkhof, P. C. M. *Br. J. Dermatol.*, 1987, *117*, 297.

[153] Wozel, G.; Chang, A.; Zultak, M.; Czarnetzki, B. M.; Happle, R.; Barth, J.; van de Kerkhof, P. C. M. *Arch. Dermatol. Res.*, 1991, *283*, 158.

[154] Mehta, K.; McQueen, T.; Tucker, S.; Pandita, R.; Aggarwal, B. B. *J. Leukoc. Biol.*, 1994, *55*, 336.

[155] Brinckerhoff, C. E.; Coffey, J. W.; Sullivan, A. C. *Science*, 1983, *221*, 750.

[156] Trentham, D. E.; Brinckerhoff, C. E. *J. Immunol.*, 1982, *129*, 2668.

[157] Kuwabara, K.; Shudo, K.; Hori, Y. *FEBS Lett.*, 1996, *378*, 153.

[158] Rosenfield, R. L.; Deplewski, D. *Am. J. Med.*, 1995, *98*, 80S.

[159] Sawaya, M. E. *J. Invest. Dermatol.*, 1992, *98*, 92S.

[160] Thiboutot, D.; Harris, G.; Iles, V.; Cimis, G.; Gilliland, K.; Hagari, S. *J. Invest. Dermatol.*, 1995, *105*, 209.

[161] Knaggs, H. E.; Holland, D. B.; Morris, C.; Wood, E. J.; Cunliffe, W. J. *J. Invest. Dermatol.*, 1994, *102*, 89.

[162] Landthaler, M.; Kummermehr, J.; Wagner, A.; Nikolowski, J.; Plewig, G.; in Retinoids; Orfanos, C. E., Ed.; Springer-Verlag, 1981, pp. 259.

[163] Goldstein, J. A.; Socha-Scott, A.; Thomsen, R. J.; Pochi, P. E.; Shalita, A. R.; Strauss, J. S. *J. Am. Acad. Dermatol.*, 1982, *6*, 760.

[164] Hommel, L.; Geiger, J.-M.; Harms, M.; Saurat, J.-H. *Dermatology*, 1996, *193*, 127.

[165] Ott, F.; Bollag, W.; Geiger, J.-M. *Dermatology*, 1996, *193*, 124.

[166] Cunliffe, W. J.; Poncet, M.; Loesche, C.; Verschoore, M. *Br. J. Dermatol.*, 1998, *139 Suppl 52*, 48.

[167] Ellis, C. N.; Millikan, L. E.; Smith, E. B.; Chalker, D. M.; Swinyer, L. J.; Katz, I. H.; Berger, R. S.; Mills, O. H., Jr.; Baker, M.; Verschoore, M.; Loesche, C. *Br. J. Dermatol.*, 1998, *139 Suppl 52*, 41.

[168] Kligman, A. M.; Grove, G. L.; Hirose, R.; Leyden, J. J. *J. Am. Acad. Dermatol.*, 1986, *15*, 836.

[169] Gilchrest, B. A. *J. Am. Acad. Dermatol.*, 1997, *36*, S27.

[170] Schwartz, E.; Kligman, L. H. *J. Invest. Dermatol.*, 1995, *104*, 518.

[171] Fisher, G. J.; Datta, S. C.; Talwar, H. S.; Wang, Z.-Q.; Varani, J.; Kang, S.; Voorhees, J. J. *Nature*, 1996, *379*, 335.

[172] Wang, Z.; Boudjelal, Mohamed, Kang, Sewong, Voorhees, John J., and Fisher, Gary J. *Nature Med.*, 1999, *5*, 418.

[173] Nagpal, S.; Chandraratna, R. A. S. *Curr. Pharm. Design*, 1996, *2*, 295.

[174] Larson, R. A.; Kondo, K.; Vardiman, J. W.; Butler, A. E.; Golomb, H. M.; Rowley, J. D. *Am. J. Med.*, 1984, *76*, 827.

[175] Lin, R. J.; Nagy, L.; Inoue, S.; Shao, W.; Miller, W. H., Jr.; Evans, R. M. *Nature*, 1998, *391*, 311.

[176] Warrell, R. P.; Frankel, S. R.; Miller, J., W. H.; Scheinberg, D. A.; Itri, L. M.; Hittelman, W. N.; Vyas, R.; Andreeff, M.; Tafuri, A.; Jakubowski, A.; Gabrilove, J.; Gordon, M. S.; Dmitrovsky, E. *N. Engl. J. Med.*, 1991, *324*, 1385.

[177] Degos, L.; Dombret, H.; Chomienne, C.; Daniel, M. T.; Miclea, J. M.; Chastang, C.; Castaigne, S.; Fenaux, P. *Blood*, 1995, *85*, 2643.

[178] Takeshita, A.; Shibata, Y.; Shinjo, K.; Yanagi, M.; Tobita, T.; Ohnishi, K.; Miyawaki, S.; Shudo, K.; Ohno, R. *Ann. Intern. Med.*, 1996, *124*, 893.

[179] Tsai, S.; Bartelmez, S.; Sitnicka, E.; Collins, S. *Genes Dev.*, 1994, *8*, 2831.

[180] Roman, S. D.; Clarke, C. L.; Hall, R. E.; Alexander, I. E.; Sutherland, R. L. *Cancer Res.*, 1992, *52*, 2236.

[181] Han, Q. X.; Allegretto, E. A.; Shao, Z. M.; Kute, T. E.; Ordonez, J.; Aisner, S. C.; Fontana, J. A. *Diagn. Mol. Path.*, 1997, 42.

[182] Dawson, M. I.; Chao, W.-R.; Pine, P.; Jong, L.; Hobbs, P. D.; Rudd, C. K.; Quick, T. C.; Niles, R. M.; Zhang, X.; Lombardo, A.; Ely, K. R.; Shroot, B.; Fontana, J. A. *Cancer Res.*, 1995, *55*, 4446.

[183] Fitzgerald, P.; Teng, M.; Chandraratna, R. A. S.; Heyman, R. A.; Allegretto, E. A. *Cancer Res.*, 1997, *57*, 2642.

[184] Elstner, E.; Muller, C.; Koshizuka, K.; Williamson, E. A.; Park, D.; Asou, H.; Shintaku, P.; Said, J. W.; Heber, D.; Koeffler, P. *Proc. Natl. Acad. Sci.*, 1998, *95*, 8806.

[185] Elstner, E.; Campbell, M. J.; Munker, R.; Shintaku, P.; Binderup, L.; Heber, D.; Said, J.; Koeffler, H. P. *Prostate*, 1999, *40*, 141.

[186] Benbrook, D.; Lernhardt, E.; Pfahl, M. *Nature*, 1988, *333*, 669.

[187] Lotan, R.; Xu, X.-C.; Lippman, S. M.; Ro, J. Y.; Lee, J. S.; Lee, J. J.; Hong, W. K. *N. Eng. J. Med.*, 1995, 1405.

[188] Xu, X.-C.; Ro, J. Y.; Lee, J. S.; Shin, D. M.; Hong, W. K.; Lotan, R. *Cancer Res.*, 1994, *54*, 3580.

[189] Bischoff, E. D.; Gottardis, M. M.; Moon, T. E.; Heyman, R. A.; Lamph, W. W. *Cancer Res.*, 1998, *58*, 479.

[190] Demetri, G. D.; Fletcher, C. D.; Mueller, E.; Sarraf, P.; Naujoks, R.; Campbell, N.; Spiegelman, B. M.; Singer, S. *Proc. Natl. Acad. Sci.*, 1999, *96*, 3951.

[191] Tyson, J. E.; Wright, L. L.; Oh, W.; Kennedy, K. A.; Mele, L.; Ehrenkranz, R. A.; Stoll, B. J.; Lemons, J. A.; Stevenson, D. K.; Bauer, C. R.; Korones, S. B.; Fanaroff, A. A. *N. Engl. J. Med.*, 1999, *340*, 1962.

[192] Massaro, G. D.; Massaro, D. *Am. J. Physiol.*, 1996, *270*, L305.

[193] Massaro, G.; Massaro, D. *Nature Med.*, 1997, *3*, 675.

[194] Oikawa, T.; Okayasu, I.; Ashino, H.; Morita, I.; Murota, S.; Shudo, K. *Eur. J. Pharmacol.*, 1993, *249*, 113.

[195] Lansink, M.; Koolwijk, P.; van Hinsbergh, V.; Kooistra, T. *Blood*, 1998, *92*, 927.

[196] Kurtz, T. W.; Gardner, D. G. *Hypertension*, 1998, *32*, 380.

[197] Chen, S.; Gardner, D. G. *J. Clin. Invest.*, 1998, *102*, 653.

[198] Miano, J. M.; Kelly, L. A.; Artacho, C. A.; Nuckolls, T. A.; Piantedois, R.; Blaner, W. S. *Circulation*, 1998, *98*, 1219.

[199] Tontonoz, P.; Hu, E.; Graves, R. A.; Budavari, A. I.; Spiegelman, B. M. *Genes Dev.*, 1994, *8*, 1224.

[200] Reaven, G. M. *Diabetologia*, 1995, *38*, 3.

[201] Clark, C. M., Jr.; Lee, D. A. *N. Engl. J. Med.*, 1995, *332*, 1210.

[202] Peraldi, P.; Xu, M.; Spiegelman, B. M. *J. Clin. Invest.*, 1997, *100*, 1863.

[203] Zhou, Y.-T.; Shimabukuro, M.; Wang, M.-Y.; Lee, Y.; Higa, M.; Milburn, J. L.; Newgard, C. B.; Unger, R. H. *Proc. Natl. Acad. Sci.*, 1998, *95*, 8898.

[204] Nestler, J. E.; Jakubowicz, D. J.; Evans, W. S.; Pasquali, R. *N. Engl. J. Med.*, 1998, *338*, 1876.

[205] Fisher, G. J.; Esmann, J.; Griffiths, C. E. M.; Talwar, H. S.; Duell, E. A.; Hammerberg, C.; Elder, J. T.; Karabin, G. D.; Nickoloff, B. J.; Cooper, K. D.; Voorhees, J. J. *J. Invest. Dermatol.*, 1991, *96*, 699.

[206] Bershad, S.; Rubinstein, A.; Paterniti, J. R.; Le, N. A.; Poliak, S. C.; Heller, B.; Ginsberg, H. N.; Fleishchmajer, R.; Brown, W. V. *N. Engl. J. Med.*, 1985, *313*, 981.

[207] Georgala, S.; Schulpis, K. H.; Potouridou, I.; Papadogeorgaki, H. *Int. J. Dermatol.*, 1997, *36*, 863.

[208] Vahlquist, C.; Olsson, A. G.; Lindholm, A.; Vahlquist, A. *Acta. Derm. Venereol.*, 1995, *75*, 377.

[209] Rizvi, N. A.; Marshall, J. L.; Ness, E.; Yoe, J.; Gill, G. M.; Truglia, J. A.; Loewen, G. R.; Jaunakais, D.; Ulm, E. H.; Hawkins, M. J. *Clin. Cancer Res.*, 1998, *4*, 1437.

[210] Vahlquist, C.; Lithell, H.; Michaelsson, G.; Selinus, I.; Vahlquist, A.; Vessby, B. *Acta Derm. Venereol.*, 1987, *67*, 139.

[211] Gustafson, S.; Vahlquist, C.; Sjoblom, L.; Eklund, A.; Vahlquist, A. *J. Lipid Res.*, 1990, *31*, 183.

[212] Vu-Dac, N.; Gervois, P.; Torra, I. P.; Fruchart, J.-C.; Kosykh, V.; Kooistra, T.; Princen, H. M. G.; Dallongeville, J.; Staels, B. *J. Clin. Invest.*, 1998, *102*, 625.

[213] Nagasaki, A.; Kikuchi, T.; Kurata, K.; Masushige, S.; Hasegawa, T.; Kato, S. *Biochem. Biophys. Res. Comm.*, 1994, *205*, 1510.

[214] Koyama, E.; Golden, E. B.; Kirsch, T.; Adams, S. L.; Chandraratna, R. A.; Michaille, J. J.; Pacifici, M. *Dev. Biol.*, 1999, *208*, 375.

[215] Standeven, A. M.; Davies, P. J. A.; Chandraratna, R. A. S.; Mader, D. R.; Johnson, A. T.; Thomazy, V. A. *Fundam. Appl. Toxicol.*, 1996, *34*, 91.

[216] DiGiovanna, J. J.; Sollitto, R. B.; Abangan, D. L.; Steinberg, S. M.; Reynolds, J. C. *Arch. Dermatol.*, 1995, *131*, 1263.